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THE JOURNAL OF PARASITOLOGY

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Harold W. Brown.

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PARASITOLOGY, PARASITIC DISEASES, AND MEDICINE*

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One of the pleasures of belonging to this Society is the opportunity of rubbing mental elbows with its members with their varied interests: taxonomists of worms, arthropods, molluses, fungi, protozoa, bacteria, and viruses; physiologists who delve into the urges and surges of these parasites, focusing on enzyme systems and individual enzymes that regulate their complex lives; chemists and pharmacologists among us seeking out new compounds that discommode or kill all of these parasites without injuring their human, animal, or plant host. Pathology, epidemiology, and clinical medicine are also our tools. All of these to what end? From a philosophic view, for the pleasure that seeking new knowledge has always given man. From the practical view, so that we may make the lives of man and his servant animals and plants, untroubled, productive and of normal span. It is this latter view, the relation of parasitic diseases to medicine, which I wish to dwell and reflect upon from an eminence of a quarter century in height.

Surveys have been made from time to time on the teaching of parasitology and tropical medicine in our medical schools. Many schools devote little or no time to the subject. It may be taught by those who have little interest or knowledge of it. Yet, according to Stoll, 2,257,100,000 persons on the globe harbor helminth parasites; and I would venture an estimate that an equal number harbor parasitic protozoa. Often, hours of medical education are devoted to an esoteric, rare clinical syndrome which has been reported from man not more than a dozen times. An occasional medical school parasitologist, an incipient empire builder, acquires a large number of hours, more than he can profitably use. In teaching parasitology to medical students I cast my vote for a delectable taste rather than a nauseating overdose. Have the student leave the course feeling that it is interesting, medically important and useful, so that 10 years after graduation when he sees an article in his sacred work, "The Journal of the American Medical Association," he will have an urge to read it, at least the summary.

Medical students and physicians are primarily interested in human biology (medicine), not zoology or parasitology. The term "parasitic diseases," therefore, more accurately describes the interests of this group and the focus of their studies. This important group of diseases, when presented as a zoological exercise with emphasis on morphology, bores medical students whose interest is in disease of man, not Laurer's canal and antepygidial bristles.

Parasitic diseases courses are usually given in the second year of the medical curriculum, an ideal time, as this is a "laboratory year" when long hours in the laboratory are readily accepted. Attempts to present the basic phases of parasitism of man to third year students when they have their stethoscopes and are on the wards is doomed to failure, and even the talented professor to ignominious defeat. During the third and fourth years, the so-called clinical years, bed-side instruction on parasitic-infected patients is acceptable to the "stethoscopic crowd." Here the focus is on clinical aspects and therapy, with a review of basic biology as it applies to the infection of the patient.

The interest of the second year student in parasitic diseases can be increased by a complete and varied loan collection of slides. The adult parasites and their several stages of development, their vectors and intermediate hosts and tissue sections demonstrating the pathologic conditions produced in man should all be present. Our parasitic diseases collection contains

* Address of retiring President, Los Angeles, California, November 3, 1960.

Editor's Note: During his presidency Dr. Brown made definite efforts in the direction of improving the Journal of Parasitology and its services to the Society. It seemed appropriate that this address be the first paper set in the newly adopted two-column arrangement as a tribute to him.

150 slides. If we are to compete successfully for the interest of the student, we must have laboratory material in abundance. We must recognize that in the second year we are competing for the students' minds with clinically oriented pathology, microbiology, pharmacology, and physical diagnosis. Human material is much preferred to animal forms. I remember our disgust as first year medical students when in our histology class collection we found a section of a spleen of a lion and heart of a salamander. Entamebae from snakes may leave medical students as cold as the invertebrate host when it is *E. histolytica* that harms man. Every effort should be made to present the students with parasitic material from human sources. Examination of their own stools is rewarding. The anatomy of parasites should be given only in sufficient detail so that the medical student can identify, with the aid of his text book, the common parasites of man. The simplicity of the parasite in comparison to its human host is stressed, in order to impress the student with the problem we face in chemotherapy; viz., eliminating a simple, cuticle-covered parasite from the intestines, blood stream, or lymphatic system of man, with a drug which is not supposed to harm man's complicated organs.

During the past quarter century careful physiological studies have given us a brief glimpse into the functioning of a few parasites. Some of their enzyme systems are now a partially open book. The LOBUND findings on the bacterial requirements of *E. histolytica* in the intestine of guinea pigs is of special interest medically, because of the importance of bacteria and their implication in chemotherapy. Microfilarial periodicity and its relation to rest, exercise, and pulmonary gaseous exchange gives an opportunity to discuss physiological adaptation of the parasite to the host and admit our ignorance of the reasons. We now have some insight into the mechanisms of immunity to parasitism, and age and racial factors are significant.

An especially interesting facet of parasitic diseases is the habitats that parasites choose in their hosts. At present it is impossible to explain the sites of predilection in man of the three schistosomes harbored or his filariae, to name just a few. Natural infection rates with various parasites vary greatly, and often the explanation is obvious. It is a matter of food, excreta disposal, arthropods, ablution, and other customs. A recent report by Davis and Read suggests that *Trichinella* infection rates in mice may be greatly influenced through the endrocrine system by the stress produced by crowding. One wonders how wide-spread this factor is applicable in the human race.

Medical students are interested in pathology for here they first come to grips with disease in man, their purpose in entering medicine. Many years ago our professor of pathology, Dr. H. P. Smith, turned over to the parasitic diseases teaching collection, from the pathology teaching collection, all of the slides that contained sections of parasites. We felt that the student would understand the pathologic lesions best if he were first versed in the parasite's anatomy, physiology, and life cycle. These are all given more easily in parasitic diseases than in a pathology course which can use all of its allotted time teaching pathology. The varying grades and types of response of the host to parasites are due to basic pathologic mechanisms. The failure of the host to respond dramatically and to show no visible evidence of injury is of special interest, for it demonstrates to the student why so many parasitic infections are subclinical.

The diagnosis of disease or the absence of it is a primary function of the physician; and, hence, the diagnosis of parasitic disease is attractive to medical students. Since many parasitic infections are asymptomatic or produce only mild symptoms, the student must develop a high index of suspicion. Routine blood and stool examinations will uncover many unsuspected infections. It is obvious that a busy clinician will not do his own laboratory examinations; but if he has made them once in class, he will know their pitfalls and accuracy. Most important, the physician should know what specimen to collect, the value of purged stools in amebiasis, the uselessness of stool examination in enterobiasis, and the necessity for repeated stool examination in amebiasis and schistosomiasis.

Recently, several instances of fatal overwhelming infections with *Strongyloides* have been reported. One patient had several hospital admissions with a chief complaint of diarrhea, dysentery, and abdominal pain. Surgery was performed several times, yet no stools were examined for ova or parasites. A toothpick, glass slide, feces the size of a small rice grain, a

microscope, and a suspicious medical student or intern could have made the diagnosis.

The availability and significance of the intradermal, complement-fixation, flocculation, and hemaglutination tests are also tools of the physician. Eosinophilia for some unknown reason is given great importance in the medical mind as a sure indication of a parasitic infection. It is true that many parasitic infections elicit an eosinophilic white cell response—notably *Trichinella*, visceral larva migrans as well as filaria, hookworm, schistosoma, and *Trichuris*, to name a few. Yet the eosinophil count may remain normal in the presence of several of these parasites as well as many others. In addition, there are a host of other causes of eosinophilia in man. Hence, either the absence or presence of eosinophilia should raise one's index of suspicion for a parasitic disease. A low total white blood cell count suggests visceral leishmaniasis and a high count may help us distinguish between viral hepatitis and protozoal and helminth liver invasion. Liver function tests are of some value both in the diagnosis of parasitic diseases and in their exclusion from the diagnosis.

We have found that slides of fresh material containing unknown helminth eggs or protozoa are a great source of stimulation to the medical students. They become quite expert in the diagnosis of malaria and helminths, and their experience with the intestinal protozoa chastens them, but gives them appreciation of the difficulty in finding and identifying them. When a stool is reported negative, though the patient has appropriate symptoms of amebiasis, their evaluation of the laboratory technician's report is intelligently critical. They appreciate the dangers of the unwary or untutored technician.

In the time alotted the medical course in parasitic diseases, students do not become expert in the diagnosis of parasitic infections any more than they become competent pathologists after completing second year pathology. They should be able, however, with the aid of a textbook to make an intelligent laboratory study of a patient's parasitic disease. Part of this training is to learn their limitations and where to turn for expert advice. Our experience has been that the common parasites, except intestinal amebae, are usually correctly diagnosed by medical students and the house staff. It is the unusual parasite, artifact, or pseudo-parasite that confuses them. Long, white mucus casts from the intestine, especially from constipated pregnant women, are brought to us as possible round worms, as are elongate strings of clotted blood passed in the urine or coughed up following pulmonary hemorrhage. Anything bizzare passed by a patient from the tropics is especially suspect, although recently a female patient from New Jersey, with no history of foreign travel, was diagnosed as having *Schistosoma hematobium*. It is true she was experiencing urinary discomfort and passing red blood cells in her urine. Desquamated epithelial cells were mistaken for the terminal-spined *hematobium* eggs.

To the physician, the usual site of the parasite in the host is necessary information, but we must not forget the parasitic peregrinations which lead to exotic signs and symptoms. Several years ago an up-state New York physician called long distance and asked about the treatment of schistosomiasis. We gave him the desired information thinking that he had under his care one of the ubiquitous Puerto Ricans. On asking if his patient were from that island, his reply was that the patient was an elderly woman who had resided in New York State all of her life except for a short excursion to Hawaii several years before. On the removal of her gall bladder a small mass was noted in the upper intestinal wall, which on sectioning contained a parasite which they diagnosed as a *Schistosoma*. Her stool was negative for ova and parasites. As this patient had never visited a recognized endemic area of schistosomiasis, we requested a section of the material for study and suggested that the antimony therapy for schistosomiasis be withheld. Here we see the parasite (fig. 1) the two suckers, its size and the spines are those of *Fasciola*. This wayward worm was causing its host no recognizable symptoms and, because of its encysted position, no ova found their way into the patient's stool. Parasites of man and their intermediate hosts are where you find them. Recently Dr. Sterman and I reported a New York City mother and son living in an apartment with a pet dog. The mother had two large abdominal echinococcus cysts and the son harbored one. The dog, which had been brought from Greece, harbored the adult worm, and ova were present in its stool.

Some years ago I was called into consultation on the obstetrics ward of our hospital. There I found two Chinese women about to be

FIGURE 1. *Fasciola* in wall of small intestine.

delivered of their babies. They had just been placed under isolation because our laboratory had reported the presence of *Fasciolopsis buski* eggs in their stools. This rather formidable name was, in the nurses' judgment, sufficient to place them under strict isolation, with all the nursing handicaps that this entails. When the necessity for fecal indifference, a snail, and water caltrops for the propogation of this parasite were presented to the nurses and intern, isolation was discontinued.

The geographical distribution of parasites aids us in diagnosis by directing our attention toward certain possibilities. However, the world is so vast and careful surveys so limited, that one must impress students that they may find the first case from a region. A number of years ago a missionary, Miss B. from Portuguese Angola, appeared at the New York City Tropical Disease Clinic with extensive skin lesions (fig. 2). Scrapings of the lesions revealed *Onchocerca* microfilariae. Miss B. then asked us if by any chance this parasite could be responsible for the gradual loss of sight of her fellow missionary who had accompanied her and was in the waiting room. Poor Miss M.'s vision was 20/400, and in spite of repeated visits to ophthalmologists in Canada and the United States nothing had been achieved. It was onchocerciasis; a nodule containing numerous adult worms was removed from her head; and she was treated with hetrazan with considerable improvement in her vision. The area where these two missionaries served was in a supposedly *Onchocerca*-free area, at least it had not been reported there. This solved the eye difficulties of a number of the missionaries, as a skin survey of the area showed the parasite to be quite prevalent. New and even extensive distribution of parasites are being reported: a focus of *S. mansoni* in the Dominican Republic, *S. japonicum* in Thailand, and recently the first case of human *S. japonicum* infection in Taiwan.

We usually associate clonorchiasis with the oriental race; however, recently German-Jewish refugees who sojourned in Shanghai for several years before coming to the United States are exhibiting *Clonorchis*. In Shanghai in 1946, some 7,000 of these refugees, whose diet was low in protein, were sold as "herring," a salt-water fish, small fish raised on human feces in fresh-water ponds by enterprising Chinese. During the past year we have had several of these patients from the Shanghai epidemic in our Medical Center. One of our surgeons in

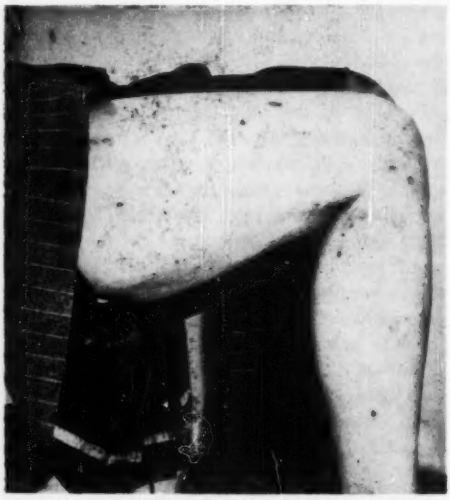


FIGURE 2. Onchocerciasis rash. Missionary from Portuguese, Angola (West Africa). Microfilariae were present in scrapings of the lesions.

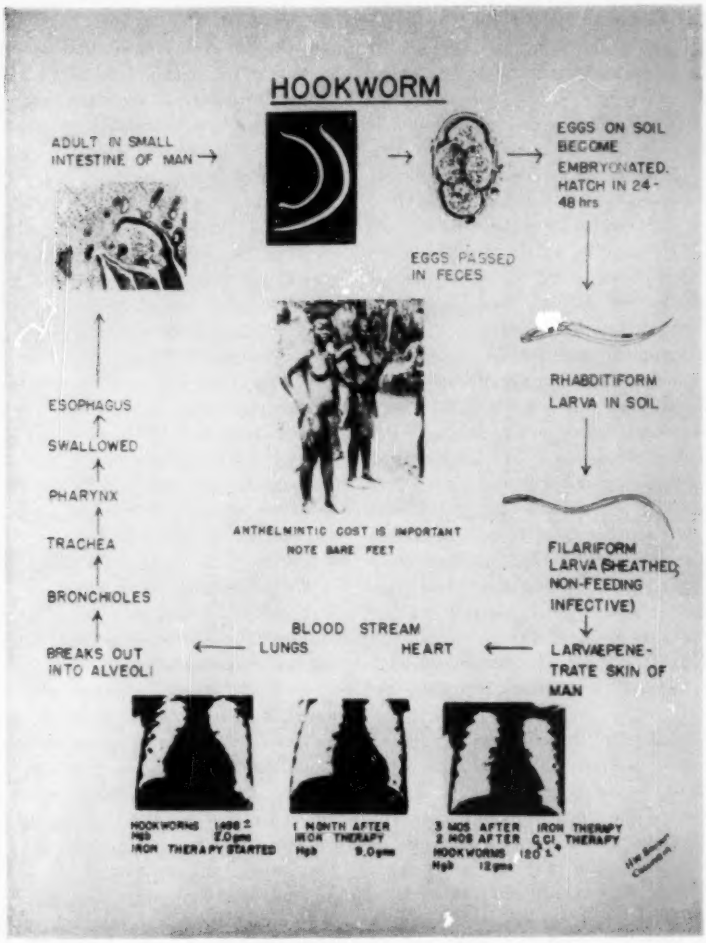


FIGURE 3. Hookworm life cycle. X-ray pictures are from Hill and Andrews, A.J.T.M., 1942.

removing a gall bladder saw "some mucus in a bile duct begin to move, and then a beady eye peered out at him,"—a full-grown *Clonorchis!*

The geographic distribution of parasitic infections is varied and knowledge of their distribution is of great value in knowing what to look for in a patient. The patient with urological problems who had been living in New Jersey all of her life could not have had *Schistosoma hematobium.* On the other hand, a Yemeni gentleman who had lived in Portland, Maine, from 1919 to 1944 was found to have far-advanced liver damage with marked ascites due to *S. mansoni* infection. He had lived in Yemen until he was 14, then spent 5 years in the British Navy prior to taking up residence in Portland. As I recall, 11 consecutive daily stools were examined before Schistosome eggs were found in his stool. His early Arabian habitat, fortunately, encouraged the medical resident to continue stool examinations.

Parasitic diseases offer an unusual opportunity to teach preventive medicine, human habits, sociology, medical economics, and epidemiology. According to Stoll (1947), 27,000,000 people in the world have *Trichinella spiralis* and 21,000,000 of this vast horde dwell in the United States and Canada. Pork is the common source of this infection; yet the pork tapeworm, *Taenia solium,* is seldom found in the United

States. Why? The only one I have seen in the past 30 years was probably acquired in Mexico. *T. solium* is not uncommon in parts of Central and South America; yet *Trichinella* infections are uncommon. Neither parasite is found in Puerto Rico. Try this so-called "thought-provoking" problem on your classes in parasitology and the answers will be revealing.

Religion and economics often dictate our food habits. In addition to Jews who do not eat pork, there are the hundreds of millions of Moslems who do not eat it—hundreds of millions of Hindus who are vegetarians. The Indian or Sepoy mutiny was due to alleged pork grease on bullets. Catholics are protected one day a week, Fridays, which is meatless. Puerto Ricans like to cook their pork to a crisp; millions of persons seldom can afford the luxury of meat, and when they do it is fish, often New England codfish; the Chinese are great pork eaters, but they are protected from its parasites by cooking it thoroughly. We Americans seem to enjoy meat while it is still hemorrhaging and in shock, and we are so well-to-do that we feed our hogs vast quantities of expensive partially cooked pork. Here are opportunities to relate parasitic infections to religions, cultures, and history.

The prevention of infection with parasitic diseases requires a knowledge of the life cycle of the parasite, especially the mode of human infection. One of the most common concerns of persons on finding that they have a parasitic infection is whether or not they are infectious to other members of their family and through what channel. Surgeons removing echinococcus cysts from the lungs or liver of their patient are concerned with the infectivity of the cyst material. Knowledge of the life cycle would make it evident to them that their infection could only take place by implantation of cyst material and that accidental ingestion, unlike trichinasis material, is not a source of infection.

From time to time the final examination in parasitic diseases contains a question that reads: Patients harboring the following parasites are on the wards of our Presbyterian Hospital. Check those which should be isolated: Hookworm, visceral leishmaniasis, *S. mansoni, Trichinella, malaria.* The sanitary status of our fine hospital, the presence of insects in it, and canabalism in New York are evidently all highly suspect, according to the number of checks we are accorded. This suggests that we should present the life cycle simply, without minutiae, and hope that the student will retain the important principles involved. We have developed a series of life cycle charts which emphasize how man is infected. Clinical and epidemiological data are included in these charts as they are intended for future physicians (fig. 3). In addition to the life cycle, attention is called to the fact that most of the people in the world do not wear shoes, and that anthelmintic cost is important. The two belles obviously do not have a nickle in their pockets. Note also the effect of the hookworm anemia on the size of the cardiac shadow.

Therapy of parasitic disease is the domain of the physician. In fact the average medical student feels that if he can diagnose, and he is usually willing to delegate this to a laboratory technician, and treat the patient, he has achieved his goal. The more socially conscious student would also like to be able to prevent infection.

The first problem is whom should we treat. A hookworm infection with 10 worms? An asymptomatic *Giardia* infection? A patient passing an occasional *S. mansoni* egg? In general, if we have a safe, effective chemotherapeutic agent, I believe all infected individuals should be treated. A few hookworms may consume little blood, but they consume it 365 days a year, including Sundays and holidays, as long as 14 years. Until a parasite is shown to be beneficial to man there is no reason to give it housing, hospitality, and to permit it to continue its asexual or sexual orgy. The object of therapy is to cure the patient, which involves the elimination of the parasite. In infections with such parasites as hookworm and schistosoma, elimination of 95 percent of the parasites may lead to the disappearance of the patient's signs and symptoms. In parasitic infections where there is a multiplication or addition of the parasite in the host, as in amebiasis and malaria (and even tapeworm), elimination of the parasite must be complete. As we reduce the host's parasite load, careful laboratory search, using proper technics on adequate specimens, at proper intervals, must be made. It may be necessary to wait weeks or months for the parasite to reappear in diagnosable numbers. Patients from whom the head of *Taenia* or *Diphyllobothrium* was not recovered must be warned that 4 to 6 months are necessary to be sure of a cure. Inci-

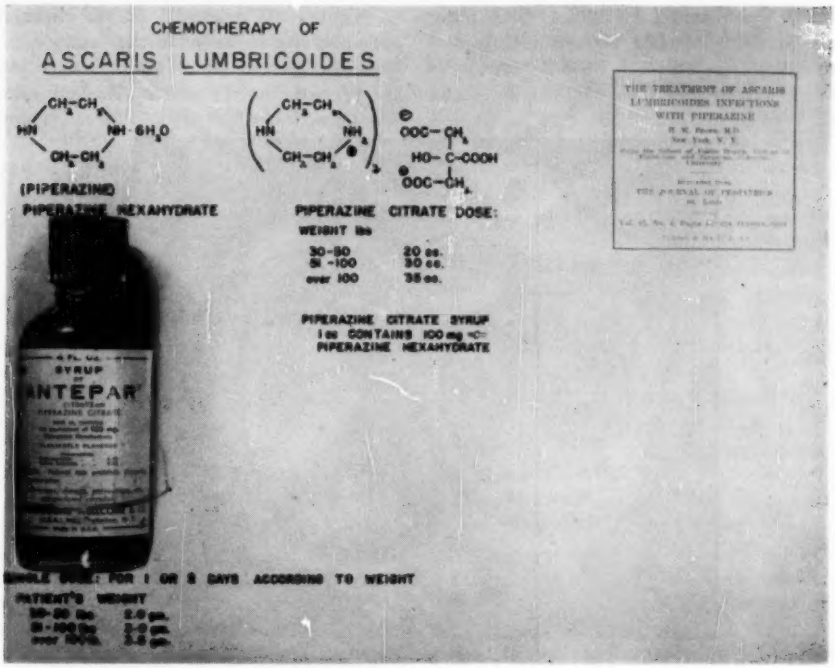


FIGURE 4. Chemotherapy demonstration chart and relevant literature.

dentally, during cestode therapy, if the toilet paper is not disposed of in the bed-pan material to be searched through for the scolex, one avoids the necessity of scanning each page for it.

The drug of choice should be stressed, giving its advantages and disadvantages. Its mode of action on the parasite can be a fascinating story, as was the demonstration of Norton and DeBeer that piperazine acts on the myoneural apparatus of *Ascaris* temporarily relaxing it. It thereby loses its urge to move upstream and to press against the sides of its host's intestine in order to maintain its position. Peristalsis carries it out in its relaxed hours.

It is a waste of time to attempt to memorize drug dosages in the second year medical course. These are much better reserved for the bedside years when the student is actually concerned with therapy. Most of us know well only those dosages that we employ clinically from time to time. It is easy and safer to look up the dosage of those used infrequently. Chemotherapy of parasitic infections has made great studies in the past 20 years. Keeping up to date is important. Although I helped Lamson develop hexylresorcinol for ascariasis 30 years ago, I now much prefer piperazine. Tetrachlorethylene, the drug of choice for hookworm these past years, has had to give up its place in the treatment of *Ancylostoma* (not *Necator*) to bephenium hydroxynaphthoate.

Chemotherapy can be a useful diagnostic tool when clinical diagnosis is impossible and when laboratory tests are unavailable or questionable. Hepatic amebiasis responds clinically and diagnostically to therapy. Chemotherapy lends itself well to further emphasis on the reason for knowing the life cycle of malaria in its exoerythrocytic and erythrocytic stages. Sometimes, students question the necessity of such detail. A new-born baby in our hospital was given a complete transfusion because of erythroblastosis and within two months it required several additional transfusions. Anemia continued along with a low grade fever. A diagnosis of hypersplenism was made and the spleen was removed surgically. Then the baby developed a classical *P. malariae* attack. Its blood teemed with the parasites. The treatment was interesting because it involved no exoerythrocytic stages, but there was no aid available in the chemotherapy from the absent spleen.

Within a short time a vacationer from Cuba with a *P. vivax* infection entered our hospital to complete our story of malaria therapy—*vivax* — exoerythrocytic — erythrocytic — and spleen were all present.

We use the students' natural interest in chemotherapy as an additional method of introducing them to the literature. Demonstrations are prepared of the various chemotherapeutic agents, part of which are reprints of important

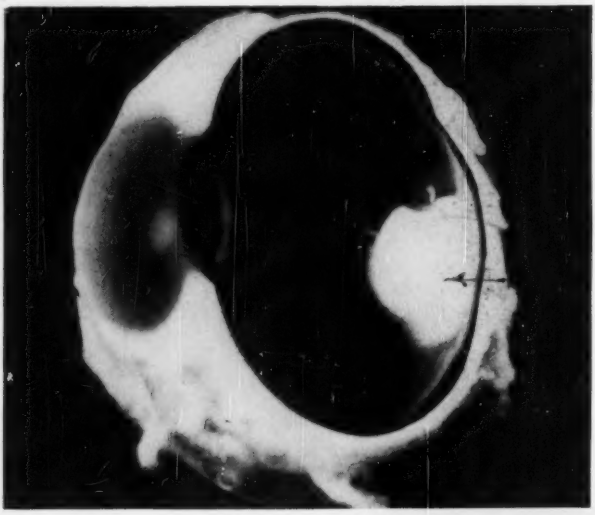


FIGURE 5. *Taenia solium* cyst in eye, gross and microscopic.

publications (fig. 4).

The practice of parasitic diseases can be integrated into the third and fourth years of medicine and the residency program. As our parasitology laboratory serves as the diagnostic laboratory for all of the hospitals in the medical center, we know immediately what parasitic infections are on the various hospital wards. We are frequently called on for additional assistance in diagnosis and therapy, giving us an opportunity to develop further the clinical aspects of parasitic diseases. These bed-side conferences are learning as well as teaching opportunities. We recently were involved in a diagnosis of a retinoblastoma vs. *T. solium* cyst of the eye of a 3-year-old child. Approximately 1 in 34,000 children are affected with retinoblastoma which makes its appearance in the first 4 years of life. This slide (fig. 5) shows the parasite and makes a definitive diagnosis. The child's residence was mistakenly given to us as Puerto Rico, but when we questioned this, it was changed to Costa Rica. *T. solium* is not found in the former, but is not uncommon in the latter.

Medical students should be given a basic background in medical entomology. As usually only a few hours are available for this extensive subject, selection of material is especially important. Arthropods that cause disease such as *Sarcoptes,* bed bugs, lice, chiggers, ticks, spiders, fire ants, and fly larvae, and the appearance of the disease produced are of special importance. The knowledge of disease vectors should be sufficient to cause the physician to search for or to question the patient about the presence of arthropods. The ability to identify vectors such as *Culicoides,* various mosquitoes, etc., in detail is for the entomologist.

Enthusiasm in students is an asset, but sometimes it requires redirection. A single, egg-like artifact in the stool of a patient with pernicious anemia is given undue prominence in the diagnosis of a diphyllobothriasis anemia. Blood platlets above or with superimposed red blood cells in patients with fever become malaria parasites. A patient with fever of lymphoma origin was given unwarranted hope by an intern for this reason. All diagnosis by the enthusiastic and partially tutored should be checked by experts. Stools that have laid around the patient's home for a day or two, insecurely covered, may present bizzare and wonderful artifacts that crawl or are wafted into the specimen. Fly maggots and arthropods are common. Recently, we were furnished only with a section of a "small worm" passed in the stool. The tissues were not diagnostic, but the teeth of the radula made the diagnosis of a gastropod, an ordinary slug.

Each year we meet to discuss research methods and results, and it is a stimulating experience for the most part. Although many of us are employed by colleges and universities whose function is *education,* and many of us are from governmental or other institutions which have some educational functions, a session, a symposium or meeting seldom concerns itself with education. When one visits a sister institution he is shown research equipment and research in action. Seldom is an exciting experiment or technic in education brought out. In fact, the research attitude of the faculty has become so widely known that students, our employers, enter our laboratories and offices with "I apologize for taking your time" or "I know you are busy," etc. Here after all is our real product, a seeking student, our opportunity, our responsibility. I would defend the value of research to every scientist and teacher, not entirely for the contributions made, but for the education research gives: and stimulation provided our students. We become sounder critics of new scientific contributions. If we gave as much thought, effort, time, and experimentation to teaching as we do to research, teaching would thrive too.

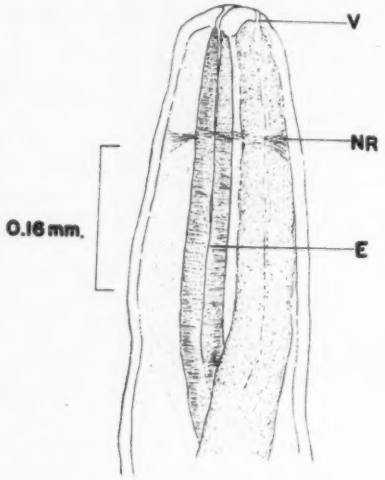
I wish to thank Dr. K. L. Hussey, my colleague of many years, for the photographs she prepared for this presentation.

RESEARCH NOTE

THE OCCURRENCE OF *FILARIA MARTIS* GMELIN, 1790, IN THE STRIPED SKUNK AND BADGER IN KANSAS.

Included in a collection of helminth parasites of Kansas wild mammals was a series of filarial worms recovered from the subcutaneous tissues of three striped skunks (*Mephitis mephitis*) and four badgers (*Taxidea taxus*). These nematodes have been identified as *Filaria martis* Gmelin, 1790, on the basis of the terminal position of the vulva, egg size and shape, and general morphological features. Records of this parasite in the skunk in the United States are very limited, and this is apparently the first report of its occurrence in the badger in this country.

F. martis, an old world species originally recorded from carnivores in Europe and North Africa (Seurat, 1920, Bull. Soc. Hist. Nat. Afr. Nord. **11**: 34–36), was reported first in the United States from the striped skunk (*Mephitis mesomelas*) and the hog-nosed skunk (*Conepatus mesoleucus*) in Texas (Tiner, 1946, J. Mammal. **27**: 82–83). These records also constitute the original report of this species in skunks. Chandler (1947, J. Parasit. **33**: 449–452) reviewed the classification of related members of the genus and described a new species, *Filaria texensis*, from the striped skunk (*Mephitis mesomelas*) in Texas. Egg size and the morphology of the egg shell were the main characters distinguishing it from *F. carvalhoi*, a South American form occurring in the skunk (*Conepatus chilensis*) in Brazil. Both American species differed materially from *F. martis* in their larger body size. Nevertheless, Caballero y C. (1948, Rev. Soc. Mex. Hist. Nat. **9**: 257–261) considered both *F. carvalhoi* and *F. texensis* to be synonyms of



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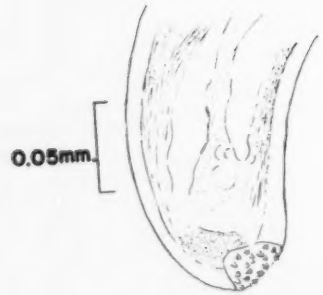

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FIGURE 1. Anterior end of female *F. martis*, showing esophagus (E), terminal vulva (V), and nerve ring (NR).

FIGURE 2. Larvated egg, cleared in glycerol.

FIGURE 3. Profile view of posterior end of female *F. martis*, showing circular mammillated area at tip of tail.

FIGURE 4. Posterior tip of male *F. martis*, showing mammillated area and caudal alae.

F. martis, to which species he assigned worms taken from a badger (*Taxidea taxus berlandieri*) collected in Nuevo Leon, Mexico.

The Kansas specimens, while resembling *F. texensis* with respect to egg morphology and spicule size, compare closely with the foregoing worms from the Mexican badger. Despite the consistently larger size of the Kansas badger filariids, the structure and dimensions of the eggs of specimens from both skunk and badger were similar, and all have been identified as *F. martis* in accordance with the classification of Caballero y C. (loc. cit.). A total of more than 40 worms of both sexes were collected in east-central Kansas (Geary, Dickinson, and Clay Counties) and in southwestern Kansas (Kearny and Hamilton Counties). One adult male skunk collected in November, 1957, yielded 1 male and 10 female worms. The other specimens from skunks, a total of three females, were recovered from two road-killed animals in March and April, 1958. An adult female badger collected in December, 1957, contained four male and four female worms. Three additional badgers collected in June and August, 1959, by Mr. M. H. Bartel, Department of Zoology, Kansas State University, yielded an undetermined number of filariids. Comparative measurements of these specimens after fixation in 70 percent alcohol or 10 percent formalin and clearing in glycerol were as follows:

	Skunk	*Badger*
Length (female)	162 mm	155–263 mm
Length (male)	111 mm	57–89 mm
Maximum width (female)	445 microns	573 microns
Maximum width (male)	259 microns	370 microns
Length of muscular esophagus	0.39–1.6 mm	0.27–1.48 mm
Maximum width of esophagus	75 microns	83 microns
Nerve ring from anterior end	171 microns	133–166 microns
Length of right spicule	166–185 microns	175 microns
Distance of anus from posterior end (female)	536–555 microns	462–665 microns
Eggs (larvated)	25–29 microns x 37–45 microns	21–25 microns x 33–41 microns

The nodular subcutaneous capsule in which many of the Kansas worms occurred was mentioned briefly in Seurat's description of *F. martis*, and by Caballero y C. (loc. cit.), but Hall (1916, Proc. U. S. Nat. Mus. **55**: 1–94) did not refer to it. Encapsulated forms were found in all three skunks and in one of the four badgers examined. These were individually enclosed in rectangular, yellowish, membranous capsules approximately 10 mm long, 5 mm wide, and a few mm thick, inside of which the convoluted adult worm lay. These encapsulated forms were situated between the skin and fascia on the chest wall, flank surfaces of the pelvic girdle, and the abdominal body wall, especially in the inguinal region. Worms were quiescent within their capsules, but became actively motile after they were dissected free in physiological saline or tap water. Gravid female worms occurred within the capsules, indicating that this may be a transient stage in the life cycle prior to release of eggs. The presence of these encapsulated forms during both spring (March and April) and early winter (November and December) suggests that they are not merely seasonal forms but normal developmental stages of the worm in both skunk and badger.

Although the life cycle of *F. martis* has not been determined, the similarity between its subcutaneous encapsulated stage and that of *Parafilaria multipapillosa*, a filariid living under the skin of horses in southern U. S. S. R. and Greece (Losev, Erokhin, and Nikanorov, 1937, Papers on Helm. 30-yr. Jub. K. I. Skrjabin: 333–345; Baumann, 1946, Wien. tierärztl. Mschr. **32**: 52–55), suggests a parallel method of transmission. These authors found that *Parafilaria* females living in skin nodules pierced the skin to release larvated eggs to the exterior. While no thorough search was made for potential intermediate hosts in this study, bloodsucking ectoparasites were found on two of the hosts infected with filariids. One flea, a female *Pulex* sp., was removed from a skunk, and several ixodid seed ticks and some unidentified fleas were recovered from the fur of one of the parasitized badgers examined during the survey.

This is contribution No. 295 from the Department of Zoology, Kansas State University, Manhattan, and Zoology Series No. 334.—D. E. WORLEY, *Department of Zoology, Kansas State University, Manhattan.* Present address: *Parke, Davis and Co., Research Division, Ann Arbor, Michigan.*

ECOLOGICAL AND SYSTEMATIC NOTES ON SOME CHIGGER-MITES OF THE GENERA *GAHRLIEPIA, TROMBICULA,* AND *EUSCHONGASTIA* (ACARINA: TROMBICULIDAE) IN MICHIGAN AND ONTARIO.

The following notes are based, for the most part, on random collections of chigger-mites made during the course of sampling various habitats for soil and litter mites. These collections

are of interest because they provide information on the habitats of unattached larvae which, in turn, presumably suggests something of the habitat of the adults. The dates of collection of unattached larvae reported here are possibly misleading as these mites were obtained by means of a modified Tullgren apparatus; it is possible that the larvae obtained hatched in the collecting funnel under the stimulus of increased warmth.

All the collections reported herein were made by the senior author.

Gahrliepia (*Walchia*) *americana* Ewing, 1942. Two larvae from woody debris from basal tree hole in large elm; each beach of Nature Sanctuary, Point Pelee National Park, Essex Co., Ontario; 19 April 1955. This is the type of habitat predicted by Loomis (1956, Univ. Kansas Sci. Bull. XXXVII, Pt. II: 1364) on the basis of host preference (squirrels) of the larvae. Farrell (1956, Proc. U. S. Natl. Mus. **106**: 113) found larvae of *G.* (*W.*) sp. in tree holes in Duke Forest, North Carolina. *G.* (*W.*) *americana* has previously been recorded from Florida (type locality), Maryland, Wisconsin, Kansas and California.

Trombicula (*Leptotrombidium*) *myotis* Ewing, 1929. One larva, data as for *G.* (*W.*) *americana*. A widespread species occurring in Canada, U. S. and Korea according to Brennan (1959, Ann. Ent. Soc. Am. **52**: 12).

Trombicula (*Miyatrombicula*) *esoensis* Sasa and Ogata, 1953. Two larvae from wood, soil and moss from base of rotted tree-stump on hillside in deciduous forest; near Metamora, Lapeer Co., Michigan; 14 April 1956. This species, described from Japan, has been recorded from Michigan, Ontario, Montana and Utah by Brennan (op. cit.: 13). The Utah record is based on *Trombicula reesi* Allred, 1958 which Brennan considers a synonym of *T.* (*M.*) *esoensis*.

Trombicula (*Trombicula*) *gurneyi* Ewing, 1937. Ten larvae from frass from subterranean galleries of the passalid beetle, *Popilius disjunctus*, from a standing dead tree; deciduous forest; east beach of Nature Sanctuary, Point Pelee National Park, Ontario; 4 November 1956. The tree from which this collection was made also harbored many five-lined skinks, *Eumeces fasciatus*, the principal host of this chigger. Specimens of *Eumeces fasciatus* and *Thamnophis sirtalis*, collected from June through September, 1953, at Pt. Pelee National Park, were commonly found to be infested with *T. gurneyi*. In addition, a single larva was found on a juvenile *Natrix sipedon* from this area.

Trombicula gurneyi exhibits geographic variation in certain of the Standard Data and Loomis (1955, Univ. Kansas Sci. Bull. XXXVII, Pt. I: 251–267) has proposed the name *T. g. campestris* for specimens from the western part of its range. This "race" was characterized by larger scutal measurements, larger AM and S, and different ecological requirements (occurring in open country rather than deciduous forests as does *T. g. gurneyi*). Unfortunately Loomis was more concerned with the characterization of subspecific entities than the description of geographic variation and the degree of concordancy of the characters studied is not clear. The status of the western members of the *gurneyi* complex seems to be an open question and present information permits interpretations ranging from the recognition of *campestris* as a separate species to the consideration of them as a series of populations characterized by step clines in selected geographically variable traits. The problem can only be resolved by a careful character by character analysis of geographic variation throughout the range of these mites. Toward that end the Standard Data of the unattached larvae reported above are given here. These characters are chosen because they can be measured accurately and because they are known to be geographically variable; this particular series of specimens is chosen because measurements based on them are free from possible error due to changes in scutal dimensions accompanying engorgement. The data are arranged as follows: Mean ± standard error; range; number of specimens. All measurements are in microns.

AW: 59.8 ± 1.32; 59 to 62; 10. PW: 71.4 ± 2.28; 68 to 76; 10. SB: 28.3 ± 0.74; 27 to 29; 10. SD(ASB + PSB): 38.4 ± 1.66; 35 to 42; 10. AP: 17.1 ± 1.22; 15 to 20; 10. AM: 26.1 ± 0.66; 23 to 29; 10. AL: 22.0 ± 1.62; 20 to 24; 9. PL: 40.1 ± 2.79; 37 to 44; 8. Number of sensillary branches: 11.1 ± 0.83; 10 to 12; 10.

Euschongastia blarinae (Ewing, 1931). Ten larvae from beneath wet, rotting deciduous log on hillside; Bald Mt. Rec. Area, Oakland Co., Michigan; 26 November 1955. This type of habitat is similar to those described by Farrell (op. cit.: 194–195) for *E. blarinae* in the eastern U. S.

Euschongastia crateris Farrell, 1956. Seven larvae from woody debris beneath cedar tree in cedar tract; near Metamora, Lapeer, Co., Michigan; 14 April 1956. Described by Farrell (op. cit.: 202–204) from *Clethrionomys* and *Peromyscus* in Pennsylvania. (Contribution from Parasitology Laboratories, No. 26 from Department of Biology, Wayne State University, Detroit, Michigan).—DONALD E. JOHNSTON, *Institute of Acarology, University of Maryland, College Park*, AND DOMINIC L. DEGIUSTI, *Wayne State University, Detroit, Michigan.*

THE LIFE CYCLE OF *DIROFILARIA UNIFORMIS* PRICE AND TRANSMISSION TO WILD AND LABORATORY RABBITS

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Filarial worm infections available for laboratory studies have heretofore been limited to forms that are taxonomically and physiologically far removed from the *Wuchereria spp.* which cause human filariasis. A laboratory infection in a convenient host with a parasite more closely related to the human forms was the object of a study conducted at the Patuxent Wildlife Research Center with the cooperation of the Fish and Wildlife Service. Attention was directed toward *Dirofilaria uniformis* Price, 1957, a parasite of the cottontail rabbit. The life cycle of *Dirofilaria scapiceps* (Leidy, 1886), a related species which occurs in a capsule in the tarsal bursa of *Lepus* and *Sylvilagus,* was elucidated by Highby (1943). He named five species of *Aedes* from Minnesota as vectors, and successfully transmitted *D. scapiceps* from the snowshoe hare to domestic rabbits. The life cycle of *D. uniformis* has been completed with the transmission to laboratory and wild cottontail rabbits, and the results are reported here.

MATERIALS AND METHODS

A wild rabbit *Sylvilagus floridanus mallurus* (Thomas) (# 309), trapped on Blakistone Island, Maryland, in September 1955, became the principal source of microfilariae for transmission experiments because it adapted well to captivity and handling.

Anopheles quadrimaculatus mosquitoes obtained from the Agricultural Research Center, U. S. Department of Agriculture, were reared in an insectary under controlled conditions of temperature and humidity. Infective blood meals from rabbits were given by inserting the rabbits' heads and forequarters through the arm hole of a 12 × 12 × 12-inch screened cage with a sliding glass front. Feeding in this manner eliminated the need for anesthesia with its attendant dangers to the animal. A second blood meal was given on a guinea pig 5 days after the infective feeding. A 10 percent sucrose solution was kept in the cage at all times.

Infective blood meals were similarly given to *Aedes aegypti, Aedes sollicitans,* and *Culex pipiens* and the mosquitoes were dissected at intervals to observe for development of the microfilariae.

The mosquitoes were dissected in 0.6 percent saline at 6-hour intervals to observe the developmental stages of *D. uniformis.* Living larvae as well as fixed and stained specimens were studied. Second- and 3d-stage larvae were fixed in Bless' fluid using a method described by Feng (1936), while earlier stages were fixed in 2 percent formalin. Wet mounts were prepared using 2 percent formalin and diluted dyes. A large number of stains were tried in an attempt to find one that would clearly define the anatomy of the larvae. Best results were obtained with brilliant green, although methyl red, toluene O, and azure II sometimes gave good results.

For transmission experiments, the 3d-stage infective larvae from *A. quadrimaculatus* were dissected out of the mosquito, counted, and injected subcutaneously into the experimental hosts. The head of each mosquito was detached from the body and the tip of the proboscis was placed in warm 0.85 percent saline. With this stimulus many of the infective larvae migrated immediately into the saline from the proboscis and head capsule. However, it was usually necessary to dissect the mouth parts of the mosquito to obtain all of the larvae present. As many as 45 larvae

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were obtained from a single mosquito by this method. All of the larvae used in the infection experiments were 9 to 11 days old and will be referred to as "3d-stage" or infective larvae through the remainder of this report.

The laboratory rabbits used in the experiments were presumably all New Zealand breed and had just been weaned, with the exception of Nos. 329, 330 and 331, which were young adults. These three also received 250 mg of cortisone acetate in five doses of 50 mg each, 2 days prior, on day of infection, on the 4th and the 11th day postinfection. The wild rabbits were mature animals that had been kept in the laboratory 4 to 6 months prior to inoculation. Weekly examinations of blood obtained by heart puncture by a modified Knott's technique (Herman and Price, 1955) were started 30 days after exposure and continued until either microfilariae were found or the animals were necropsied. With animals that remained negative 120 days after exposure, the examination was made late in the afternoon or evening in order to detect possible light infections.

RESULTS

Observations on Development in the Insect Vector

The microfilaria and adults of *D. uniformis* were described by Price (1957). The developmental stages of this parasite have been found to be similar to those of other filariids, e.g., *Wuchereria malayi,* described by Feng (1936), and *Ornithofilaria fallisensis,* described by Anderson (1956). Upon ingestion by the *Anopheles quadrimaculatus,* the majority of the microfilariae shed their sheaths in the stomach and moved through the stomach wall into the hemocoele of the abdomen. Twenty-four hours after ingestion by the mosquito (plate I, fig. 1), the developing larvae were still very active and motile. Despite a slight shortening and thickening, they still resembled the microfilaria. After 48 hours in the mosquito, the larvae were no longer active. A period followed in which there was a rapid thickening of the larvae and in 72 hours they had reached the "sausage stage," characterized by the prominence of the excretory cell, an anal plug, and the presence of a curved tail-like appendage (plate I, fig. 2). Fixation frequently caused the anal plug to burst (plate I, fig. 2). The tail-like appendage was lost during the first molt, which usually occurred on the 4th or 5th day of development. An advanced 2d-stage larva is shown in plate I, figure 3. It is twice the length of the sausage stage of the 3d day. The esophagus and intestine are well developed and the larva illustrated is about to molt for the second time (note separation of cuticle). No significant change in appearance occurred after molting, so the infective stage is not illustrated. However, early in this (7-day) stage the larvae became very active and left the hemocoele of the abdomen. They migrated rapidly through the thorax on the 8th day and reached the head and proboscis on the 9th day. When the tip of the proboscis was placed in warm saline these larvae migrated rapidly from the labella into the solution. Development to the 3d-stage occurred in 7 to 9 days at an average temperature of 75 F and an average relative humidity of 78 percent.

No development was observed in *Aedes aegypti, Aedes sollicitans,* or *Culex pipiens,* and all worms were dead by the 3d day after ingestion.

Experimental Transmission to Wild Rabbits

The results obtained from subcutaneous injection of infective larvae in 14 wild rabbits are summarized in table I. Ten of the animals lived until the worms matured and microfilariae were detected in the blood. The prepatent period was shortest, 97 days, in the animal (No. 327) that received the greatest number of larvae (354). It was longest, 222 days, in the rabbit (No. 322) that received the

TABLE I. *Results of exposure of wild rabbits to subcutaneous injections of the infective larvae of* Dirofilaria uniformis.

Rabbit No.	Date inoc.	No. larvae	Date positive	Prepatent period	Worms recovered	Percent recovered
304	21 July 56	65	7 Dec. 56	139 days	19	25
305	21 July 56	63	16 Jan. 57	179 days	11	17
319	14 May 56	12	—	died	None	0
320	11 June 56	20	—	died	4	20
321	11 June 56	5	—	died	None	0
322	27 June 56	(9)	4 Feb. 57	222 days	*	—
323	20 Feb. 57	(314)	18 June 56	118 days	*	—
324	20 Feb. 57	(200)	11 July 56	141 days	*	—
326	20 Feb. 57	(203)	7 June 57	107 days	*	—
327	13 Mar. 57	(354)	18 June 57	97 days	*	—
328	28 July 56	66	3 Feb. 57	190 days	13	19
332	14 Sep. 56	60	—	died	3	5
333	15 Sep. 56	122	4 Feb. 57	142 days	56	45
340	5 Oct. 56	(115)	7 Feb. 57	125 days	*	—
Total larvae in necropsied rabbits		413		Total	106	25

* Not necropsied.

fewest (9). Immature worms were found in two of the four animals that died 36 and 46 days after exposure. These worms were quite small and difficult to see in the subcutaneous tissue.

Two rabbits died shortly after inoculation. Six of the 10 rabbits with a microfilaremia also died. Three of these (Nos. 304, 305, and 328) were mature when trapped and had been in the laboratory nearly 2 years. It is believed that they died of old age. More than 25 percent of the infective larvae (413) given to these animals were recovered as adult worms (106).

Experimental Transmission to Laboratory Rabbits

Twenty-seven laboratory rabbits were exposed to infective larvae by subcutaneous inoculation. The first nine animals were given from 9 to 18 infective larvae and there was no evidence of infection after repeated blood smears or at necropsy. These animals have been omitted from the summary of results given in table II. Subsequent experiments showed that it was necessary to give larger numbers of larvae to obtain an infection in laboratory rabbits. The remaining 18

TABLE II. *Results of exposure of laboratory rabbits to subcutaneous injections of the infective larvae of* Dirofilaria uniformis.

No.	Date inoc.	No. larvae	Date positive	Prepatent period	Worms recovered	Percent recovered
329	18 Aug. 56	46	—	—	7	19
330	18 Aug. 56	61	—	—	None	0
331	24 Aug. 56	77	—	—	2 dead**	2
334	2 Oct. 56	100	—	—	1 dead**	1
335	2 Oct. 56	(179)	7 Feb. 57	128 days	*	—
336	2 Oct. 56	(125)	15 Feb. 57	136 days	*	—
337	5 Oct. 56	(137)	15 Feb. 57	125 days	*	—
341	29 Nov. 56	(160)	7 Feb. 57	110 days	*	—
342	3 Dec. 56	105	—	—	4 dead**	4
343	26 Feb. 57	317	—	—	None	0
344	26 Feb. 57	312	—	—	1 (alive)	<1
345	7 Mar. 57	386	—	—	5 dead**	2
346	10 Mar. 57	316	—	—	None	0
348	13 Mar. 57	341	—	—	9, 1 alive**	3
349	19 Mar. 57	(466)	8 July 57	111 days	*	—
350	21 Mar. 57	(321)	8 July 57	109 days	*	—
351	27 Mar. 57	(1,237)	18 July 57	113 days	*	—
352	29 Mar. 57	(507)	8 July 57	101 days	*	—
Total larvae in necropsied rabbits		2,061		Total	30	1

* Not necropsied.
** Also calcified remains in cysts.

rabbits received from 46 to 1,237 infective larvae, the number given depending upon the larvae available at the time of exposure. Eight of these rabbits developed microfilaremia and the prepatent period ranged from 101 to 136 days.

The other 10 animals received up to 386 infective larvae each, but microfilaremia did not develop. Upon necropsy, worms were found in seven of these rabbits but three were apparently negative. The animal (No. 329) which received the smallest number of infective larvae was necropsied 350 days after exposure. One adult male and six adult female worms were found. Only one of the females was fertile. A concentration of 2 ml of heart blood taken just before necropsy had not shown microfilariae.

Necropsy of the remaining six rabbits revealed evidence of host reactions against the parasites. Only two animals had living worms, one female in each rabbit (Nos. 344 and 348). Several dead worms and many small, yellowish, cyst-like pustules were found in five of the six animals. In some of the pustules the structure of a dead worm was clearly visible and others contained worms in various stages of disintegration. These worms were well developed and may have reached maturity before encapsulation and disintegration took place. Of the total larvae (2,061) inoculated into rabbits which were necropsied only 1 percent (30) were recovered as adults.

Periodicity of the Microfilariae

Periodicity studies were done on two laboratory rabbits and on two wild rabbits with experimental infections of *D. uniformis*. Thick smears were made of two 0.02-ml samples of ear blood taken at 4-hour intervals over a period of 72 hours. The blood smears were dried, dehemoglobinized, and stained with methyl green pyronin x using the method of Feng (1933). The average number of microfilariae was determined from the two slides and the results shown in figures 1 (laboratory) and 2 (wild). In the individual animals, there was little or no variation between the different 24-hour periods. It will be noted that the pattern from each animal differed somewhat from its partner in the experiment and that there were similar differences between the laboratory and wild rabbits. In general, microfilariae were present in the greatest numbers between 4:00 p.m. and midnight. It is of interest that the evening peaks correspond closely to the biting periods of *A. quadrimaculatus*.

Development of the Microfilaremia in Infected Wild and Laboratory Rabbits

Four experimentally infected wild rabbits were used in studying the development of the microfilaremia. Two 0.02-ml samples of ear blood were obtained from each rabbit at approximately 2:00 p.m. The method of preparation and examination of these samples was the same as for the periodicity study. The results of these weekly bleedings are presented in figure 3.

The course of the infection was similar in rabbits Nos. 304, 305, and 340. The initial counts were low (only two or three microfilariae per 0.02 ml and a sharp increase occurred between the 9th and 12th weeks. Rabbit No. 305 died during the 15th week, and at necropsy 13 adult female and 6 adult male worms were recovered. At necropsy there was no evidence of pathology due to the infection among the rabbits that died during the experiment. Two of the three animals that died had been in the laboratory for 2 years and their death was attributed to old

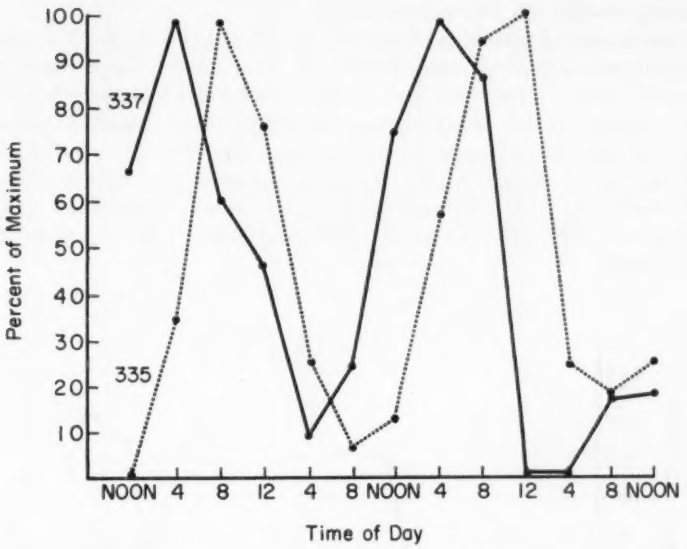

FIGURE 1. The periodicity of microfilariae of *Dirofilaria uniformis* in experimentally infected laboratory rabbits.

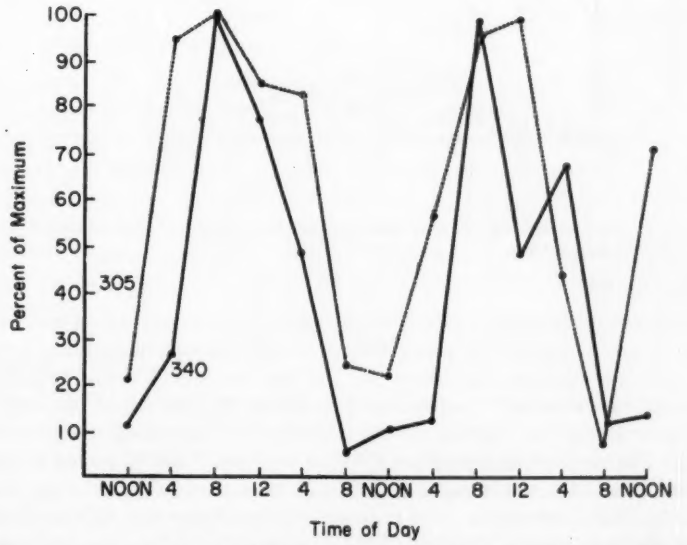

FIGURE 2. The periodicity of microfilariae of *Dirofilaria uniformis* in experimentally infected wild rabbits.

age. Death of rabbit No. 333 was accidental.

The developmental pattern exhibited by rabbit No. 333 in figure 3 is representative of that encountered in heavy infections. This animal had a much heavier infection than did the other three, and observations indicate that there is an inverse ratio between the number of worms and the length of the prepatent period. In animals with light infections, the prepatent period was several weeks longer than in those with heavy infections. The decline in the number of microfilariae which occurred during the 2d and 3d weeks after their detection is similar to a phenomenon which was observed by Anderson (1956) in ducklings infected with *Ornithofilaria fallisensis*.

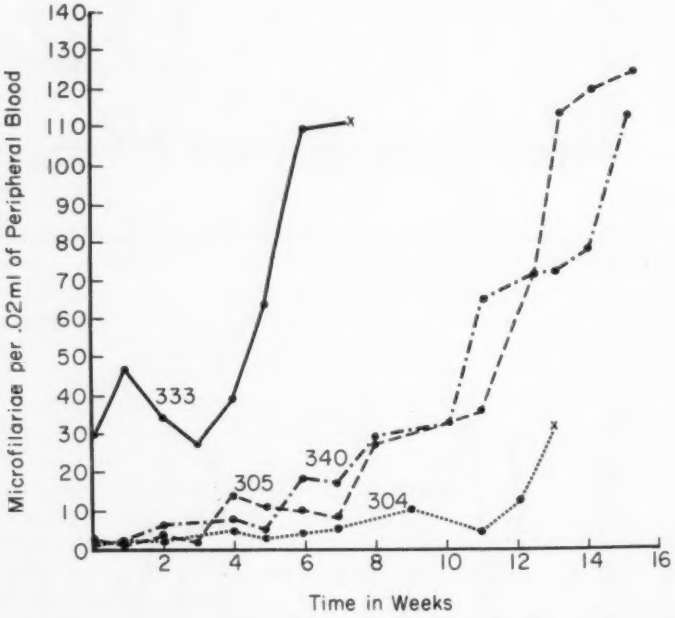

FIGURE 3. Development of the microfilaremia produced by *Dirofilaria uniformis* in experimentally infected wild rabbits.

The course of infection in the two laboratory rabbits employed was not followed as closely as that in the wild rabbits. Weekly samples were taken from No. 336 for the first 10 weeks and from No. 337 for the first 7 weeks following the detection of microfilariae. A check on No. 336 at 30 weeks and No. 337 at 26 weeks showed that the number of microfilariae had increased in both animals (fig. 4). The number of microfilariae found was small, but it should be remembered that these counts were made at 2:00 p.m. near the low point in the periodicity curve. The development of the microfilaremia apparently follows a similar course in the two species. Transfer of both authors on military orders precluded observations for a longer period.

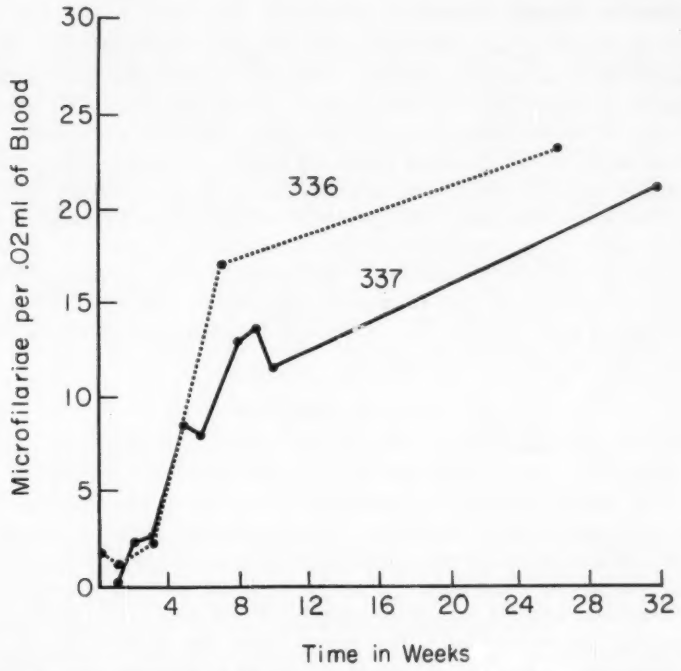

FIGURE 4. Development of the microfilaremia produced by *Dirofilaria uniformis* in experimentally infected laboratory rabbits.

Duration of Infection with D. uniformis

The number of microfilariae found in a naturally infected wild rabbit with a light infection remained at the same level for more than 2 years. The heavily infected wild rabbit (No. 309) had a microfilaria count of 672 per 0.02 ml of peripheral blood at 12:00 noon, 3 months after the microfilariae were first detected in the blood. One year later the count had risen to 1,132. Of the experimentally infected animals, the course of infection was followed the longest in a wild rabbit (No. 340). Over a period of 7 months following the detection of microfilariae, the count increased from 3 to 148. The data indicate that infections are relatively long lived.

Distribution in the Host

A total of 733 *D. uniformis* was recovered from experimentally infected wild and laboratory rabbits and naturally infected wild rabbits. All of these worms were recovered under the skin (plate I, fig. 4) of every region of the body except the head, the majority of the worms being found in the posterior half of the body. No adult worms were recovered from the tarsal bursae. However, all of 28 adult *D. scapiceps* recovered from three cottontails shot on the Patuxent Wildlife Research Center were found in the tarsal bursae.

Transplantation of Adult Worms

The location of *D. uniformis* in its host suggested that the adults might be transplanted from one rabbit to another. Necropsy of the rabbit used as the principal source of microfilariae for transmission experiments (No. 309) resulted in the recovery of 16 adult females and 9 adult males. Attempts were made to transplant these worms to two cottontail rabbits by making an incision through the skin in the lumbar region of each animal and then raising the skin slightly with a blunt probe. The worms were placed in this pocket and pushed as far away as possible from the incision. Upon completion of the transfer of the worms, the incision was sutured and painted with merthiolate. One animal received 4 female and 3 male worms, and the other received 12 females and 6 males. Daily examinations remained negative until the 20th day after transplantation, when microfilariae were observed in the ear blood of both animals.

DISCUSSION

The locality and means of acquiring infection could not be absolutely determined in the rabbit (No. 309) which was the principal source of microfilariae in this study. This animal showed no microfilariae upon several examinations between the time of its capture on 15 September 1955 and 30 April 1956. However, on 29 September 1955 it was subjected to irradiation by radioisotope tagging of its blood with Cr^{51} in another experiment. Five ml of blood was withdrawn from the marginal ear vein, citrated, and 50 microcuries of Cr^{51} were added and allowed to stand at 22 C for 30 minutes. The tagged blood was then reinjected via the marginal ear vein. No untoward effects on the rabbit were apparent, although its blood was highly radioactive as evidenced by readings of up to 292 counts per minute on a high background scintillation counter elicited from mosquitoes which had taken a blood meal. On 27 March 1956 this rabbit was injected subcutaneously with five infective larvae from *A. quadrimaculatus*. Thirty-two days later microfilariae were detected in the blood. There are thus three possibilities as to the source of this infection: (1) Acquired in nature with a prepatent period of over 7 months; (2) acquired accidentally in the laboratory during the winter months; (3) acquired as the result of experimental inoculation with a prepatent period of 32 days. The possibility of an accidental infection during the winter is extremely remote. The prepatent period of only 32 days is not consistent with the results obtained in the other experimental infections, but the possible effects of the exposure to Cr^{51} on the host are unknown. One rabbit (No. 322) in the experimental series had a prepatent period of 222 days. Assuming that No. 309 was exposed the day prior to his capture, the resulting prepatent period of 229 days would approximate this period and the explanation of a natural infection would be the most likely possibility.

Highby (1943) recovered 176 adult *D. scapiceps* from snowshoe hares in Minnesota. With the exception of one worm found below the connective tissue posterior to the tarsal joint, all were in the tarsal bursae. One of 14 *D. scapiceps* recovered by Price (1957) from *Sylvilagus floridanus mallurus* was found near the knee joint and the remainder were in the tarsal bursae. Penner et al (1953) reported *D. scapiceps* from *Sylvilagus floridanus* in New England and reviewed reports of filarial worms from rabbits in the United States. They mentioned that Hall

(1916) reported a collection by Douthitt in which filarial worms were found in *Sylvilagus floridanus alacer* from Sulphur, Oklahoma. The parasites in this case were found under the skin in the lumbar region. While the morphological differences between *D. uniformis* and *D. scapiceps* are obvious since Price's studies, a cursory examination of filariae from a rabbit could lead to confusion of the species. In the light of our present knowledge of the site of the parasites in the definitive host, it appears that the worms found by Douthitt were probably *D. uniformis*. If this is true, it indicates that this parasite has a wide geographic distribution.

The excellent results obtained by mechanical transmission of the infective larvae to wild rabbits indicated that the parasite can be maintained in the laboratory. The natural resistance of the laboratory rabbits as indicated by the much smaller recovery rate and the rapid death of the parasite prevents their use in place of wild rabbits. However, the possibility of overcoming this resistance or the discovery of a strain which is not so refractory present the prospect of this parasite fulfilling the sought-after goal of a laboratory-maintained filarial infection more closely related to the human disease agent.

SUMMARY

1. Ingested microfilariae of *Dirofilaria uniformis*, a parasite of *Sylvilagus floridanus mallurus*, developed in the hemocoele of *Anopheles quadrimaculatus* and the infective larvae migrated to the proboscis on the ninth day. No development was observed in *Aedes aegypti*, *Aedes sollicitans*, or *Culex pipiens*.

2. Subcutaneous injections of infective larvae produced experimental infections that developed a microfilaremia in 12 out of 14 wild rabbits and 8 out of 18 laboratory rabbits.

3. The length of the prepatent period was inversely proportional to the number of infective larvae administered. Microfilariae first appeared in the blood between 97 and 222 days after inoculation.

4. The microfilaremia increased in intensity for several months after the prepatent period. There was evidence that the infection may be retained for more than 2 years in wild rabbits.

5. Periodicity studies showed that the heaviest microfilaremia occurred between 4:00 p.m. and midnight.

6. To obtain infections in laboratory rabbits, it was necessary to inject larger numbers of larvae than in the cottontail. This, and the fact that in some laboratory animals the worms were encapsulated and destroyed, indicates that this host is more refractory to infection.

ACKNOWLEDGMENTS

The support of Dr. Donald B. McMullen, Chief of the Department of Medical Zoology, who supervised the project and assisted in the preparation of the manuscript, is gratefully acknowledged. We wish to express our appreciation to Dr. Carlton M. Herman of the Patuxent Wildlife Research Center and his staff, whose cooperation and assistance made the project possible. We are also indebted to the Medical Audio-Visual Department, WRAIR, for the photography and graphs, and to Mr. P. M. Bauman, Mr. J. I. Bruce, Mr. A. E. Dennis, and other members of the Department of Medical Zoology, for technical assistance.

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RESEARCH NOTE

VALLONIA PULCHELLA, AN EXPERIMENTAL SNAIL HOST OF *LYPEROSOMUM MONENTERON* (PRICE AND McINTOSH, 1935) (TREMATODA: DICROCOELIIDAE).

Lyperosomum monenteron, described by Price and McIntosh (1935, Proc. Helm. Soc. Wash. **2**: 63–64), is a dicrocoeliid trematode of the gall bladder and bile duct of the robin, *Migratorius turdus*, and the bluebird, *Sialis sialis*.

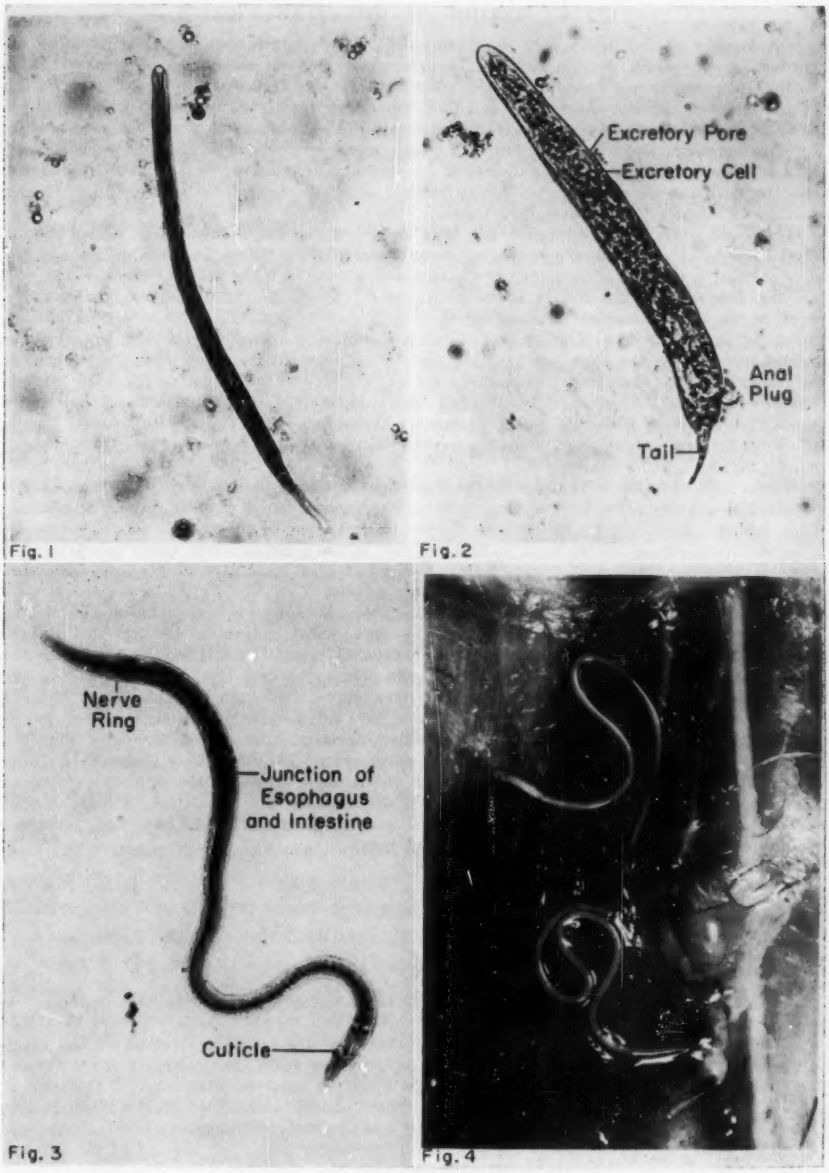
During the period 1952 to 1959, twelve robins found dead on the lawns and roads in the vicinity of Ann Arbor were collected and examined for *L. monenteron*. The range of infection in individual robins was 2 to 30 mature flukes.

In the spring and summer of 1959, an attempt was made to identify the snail intermediate host of *L. monenteron* in nature. A survey of land snails was made in various habitats where robins were observed to nest and feed. There were examined seven species of snails belonging to the families Endodontidae, Polygyridae, Zonitidae, Limacidae, Succineidae, and Valloniidae. During this study larval dicrocoeliids were found in three species of land snails, viz, *Ventridens ligerus*, *Zonitoides nitidus*, and *Succinea ovalis*. Of 3349 specimens of *Ventridens ligerus* examined, 12 were infected with dicrocoeliid sporocysts and cercariae. Of 362 *Zonitoides* examined, one was infected with sporocysts bearing long-tailed dicrocoeliid cercariae. In a lot of 363 specimens of *S. ovalis* examined, one harbored immature sporocysts of a dicrocoeliid.

On May 20, 1960, three adult flukes of *L. monenteron* were removed from the gall bladder of a robin. Mature eggs were teased from the uteri of these worms and fed to laboratory-raised specimens of *Ventridens ligerus*, *Zonitoides nitidus*, *Anguispira alternata*, *Deroceras laeve*, *Retinella indentata*, *Triodopsis multilineata*, and *Vallonia pulchella*. These molluscs were then examined for the presence of sporocysts, beginning on the 60th day after exposure of the snails to fluke eggs. Daughter sporocysts containing long-tailed cercariae of *L. monenteron* developed only in the small snail, *Vallonia pulchella* (Müller). Sporocysts did not develop in *Ventridens ligerus* or *Zonitoides nitidus*. This suggests that the natural infections found in these snails represent other species of dicrocoeliids.

Several hundred specimens of *Vallonia pulchella* and the closely related *Vallonia costata* have been collected and examined from areas inhabited by robins. To date, attempts to find natural infections of *L. monenteron* in these snails have been unsuccessful. Appreciation is expressed to Dr. Henry van der Schalie of the Department of Zoology for his kind assistance in the identification of the molluscs.—JOHN B. VILLELLA, *Phoenix Memorial Laboratory, University of Michigan, Ann Arbor.*

PLATE I



EXPLANATION OF PLATE I

FIGURE 1. Larva from hemocoele of *A. quadrimaculatus* 24 hours after ingestion, fixed in 2-percent formalin, azure II stain (×336).

FIGURE 2. Sausage stage, 72 hours after ingestion, fixed in 2-percent formalin, methyl red stain (×316).

FIGURE 3. Six-day larva (second stage), fixed in 2-percent formalin, methyl red stain (×172).

FIGURE 4. Adult worms in situ after removal of skin.

RESEARCH NOTE

A SIMPLIFIED METHOD OF ISOLATING *ASCARIS* EGGS.

A number of techniques have been employed by different investigators for isolating and harvesting eggs from *Ascaris* females. In general, these techniques involve resection of the uterus, the squeezing or maceration of the tissue to liberate the eggs followed by the passage of the material through cheesecloth and final separation by centrifugation. Various modifications of this procedure have been employed. However, the important step which I have modified involves the method of separating the eggs from the uterine tissue. The previous methods result in a relatively small yield of isolated eggs due to the large number that remain imbedded in the uterine tissue. As a result, the concentrations of eggs obtained are relatively low in comparison to the new method described below.

The procedure described below has been employed for the isolation and preparation of decoated eggs of *Ascaris lumbricoides* var. *suum* obtained from swine at a local slaughterhouse. The uteri of a number of females are resected and placed in 0.5 N NaOH. The resected uteri are transferred to a Ten Broeck tissue homogenizer (Pyrex No. 96580 small). The clearance between the ground mortar tube and pestle is 0.12 to 0.17 mm. The uteri are then homogenized by hand operation. The uterine tissue is completely disrupted resulting in a cell-free suspension whereas the eggs are liberated and left unharmed. By leaving the pestle inserted in the mortar, the homogenate containing the eggs is poured off while undisrupted material remains in the bottom of the tube. The eggs are completely decoated by repeated centrifugations at low speeds with 0.5 NaOH (generally three such washings are sufficient) Further washings by centrifugation with distilled water will completely free the eggs from contaminating material.

This method takes advantage of the condition in which the eggs are quite resistant to mechanical disintegration by grinding, whereas the uterine tissue is quite fragile and susceptible to homogenization by the procedure. Motor-driven homogenization is not recommended since it damages many of the eggs. In fact, we now utilize a motor-driven tissue grinder (Duall type, Kontes K-88545) to obtain cell-free homogenates of eggs harvested by the procedure described above.

There are several advantages of this procedure. The eggs are left unharmed as demonstrated by microscopic examination of the suspension, embryonation of the eggs to the infective stage, and infection of rabbits with embryonated eggs. The cultures harvested by this method are virtually free from uterine tissue and debris, the extent of purity depending only on the procedure of washing by repeated centrifugations. Although the description above is for the preparation of decoated eggs, uterine eggs (not decoated) can be prepared by the use of other suspending media. The homogenizing alone will not decoat the eggs. This procedure allows for the harvesting of eggs in numbers not readily feasible or accessible by other methods. A comparison of this procedure with the method involving the milking of resected uteri is presented in the table below and will demonstrate this advantage. Except for the method of separation of the eggs from the uterus, all steps were identical and performed simultaneously for the preparation of decoated eggs.

Method	*Number of worms used*	*Final volume of packed eggs*	*Total number of eggs*
Homogenizing	25	4.0 ml	88,500,000
Milking	25	2.2 ml	54,500,000

In addition, the milking procedure required twice as much time for the separation of the eggs from the uterus.

Technicians in this laboratory using only the most distal one-inch region of the uterus routinely prepare cultures containing over 20 million eggs from approximately forty females. The average time for the complete procedure is about 90 minutes, with most of the time involved in resection of the uteri. Preparations from one-inch sections of uteri of 100 females result in as much as 20 ml of packed eggs after washing and centrifuging.

This procedure will permit studies of the biochemistry and other aspects of the development of the egg previously not feasible. In addition, the operation which requires no special training or technology will provide a simplified and rapid procedure of routinely harvesting eggs.—L. C. COSTELLO, *Department of Physiology, School of Pharmacy, University of Maryland, Baltimore.*

INFECTIONS OF *TRICHINELLA SPIRALIS* IN HIBERNATING HAMSTERS*

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This paper presents the results of experiments on the effect of hibernation upon the development of *Trichinella spiralis* in the golden hamster, *Cricetus auratus*. The phenomenon of hibernation in the golden hamster has been extensively studied, and many physiological and biochemical changes associated with hibernation have been recorded (Lyman and Chatfield, 1955; Lyman, 1958).

Animals which hibernate provide an environment for their parasites different than that provided by homiotherms or poikilotherms. The body temperature of the hibernator varies, usually following a seasonal pattern, with periods of hibernation during which the body temperature may drop to a few degrees above zero centigrade (Lyman and Chatfield, 1955). Poikilotherms undergo seasonal and daily variations in body temperature, but the upper limits reached are generally lower than those of hibernators or homiotherms. Some poikilotherms may reduce the extent of daily and seasonal fluctuations by specific activity patterns (Bogert, 1959). Bats, whose body temperature drops to within a few degrees above ambient temperature except during activity, constitute a fourth category of host-temperature patterns (Hock, 1958).

Blanchard and Blatin (1907) infected hibernating marmots with three species of trypanosomes and with *T. spiralis*. They concluded that hibernation of more than 4 to 5 days would provide protection against the lethal effects of trypanosome infection if the hibernation occurred within the first week following inoculation. Three marmots were infected with trichina. One marmot was held at room temperature and developed a normal infection. The other two were allowed to hibernate after the infection, the two animals hibernating for 31 and 21 days. Neither contained trichina larvae when examined 47 and 63 days after infection. It should be noted that these animals were hibernating just before infection and resumed or continued hibernation after infection almost without interruption.

Blanchard (1903a) found that the lethal effect of *Trpanosoma bruci* in marmots was slowed by hibernation and felt that hibernation freed these animals of their parasites in nature (Blanchard, 1903b). The latter conclusion was based upon failure to find any intestinal parasites in over 100 marmots captured during hibernation.

Simitch and Petrovitch (1953) found that European ground squirrels, *Citellus citellus,* originally infected with *Hymenolepis nana,* contained no worms after hibernation in the laboratory. During a winter's exposure to cold, individual *Citellus* hibernated from 50 to 103 days. Periods of uninterrupted hibernation

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between the frequent arousals characteristic of hibernators ranged from 17 to 31 days.

By inducing hibernation in *Citellus citellus* naturally infected with various species of parasitic helminths of the alimentary tract, Simitch and Petrovitch (1954) demonstrated that the length of time required to eliminate helminth parasites varied from species to species. *Gongylonema longispiculum* withstood 30 or more days of hibernation. *Hymenolepis nana* was eliminated after 10 days of hibernation, while *H. diminuta* var. *citelli* (nomenclature is that of cited authors) could withstand 20 to 25 days of uninterrupted hibernation. The seasonal distribution of the parasites in naturally infected hosts was found to correspond with laboratory results. The more resistant species were found throughout the year; the less resistant very rarely or not at all during and after the hibernation period.

In a preliminary note on the effect of hibernation upon trichina infections in the hamster, Chute and Lewis (1958) reported that hibernation reduced the numbers of muscle larvae developing in infected hamsters. The data supporting this note are presented as experiment 1 in this paper. Chute and Covalt (1960) reported that hibernation at 5 C or continued exposure of the host to temperatures between 23 and 26 C prevented the development of adult trichina in bats.

MATERIALS AND METHODS

Fifty- to 80-gram hamsters were purchased from a commercial supplier and kept in individual wire cages. In the cold room, coffee cans full of shavings were provided for hibernation. Food in excess of daily consumption was provided in order to allow hamsters to hoard (Lyman, 1954). Many of the animals proved to be heavily infected with *Hymenolepis diminuta*. Mortality among these infected animals was very high following exposure to cold.

Temperature in the cold room was maintained at 5 C plus or minus one degree. Relative humidity in the cold room varied from 90 to 97 percent. The room was in total darkness except for two 10-minute periods in the morning and afternoon when the animals were examined and fed and for shorter periods at irregular intervals.

Temperature in the animal room, hereafter referred to as the warm room, varied widely depending upon the season. Extremes were 13 and 31 C. Relative humidity fluctuated from 60 to 80 percent.

The strain of *Trichinella spiralis* used was originally obtained from the Communicable Disease Center of the U. S. Public Health Service. It has been maintained in this laboratory in white rats. In the first two experiments the larvae were isolated from rats; in the 3d and 4th experiment from hamsters which had been infected with the larvae used in experiment 1. The methods of isolating, counting, and administering the larvae have been described previously (Chute, 1956). When negative results were recorded from an animal which had received an inoculating dose, all of the sediment from the 2 liters of digestate was examined in addition to the two routine 50-cc samples.

Animals which were hibernating on the day of infection were moved to the warm room and allowed to arouse fully before infection. The animals in the cold room were observed in the morning and late afternoon to determine if they were active, sleeping, or hibernating. Hibernation was distinguished from sleep on the basis of respiration rates. In hibernation the rates were on the order of four per minute and quite irregular.

RESULTS

Experiment 1. A group of 15 hamsters which had been in the cold room for 30 days was infected with 540 trichina each. Six of the hamsters were kept in the warm room, nine were returned to the cold room. One animal in each group died on the third day after infection; both contained numerous developing and mature trichina.

The remaining animals were sacrificed between the 33d and the 58th day of infection. The numbers of days the cold-room animals were observed to be hiber-

nating and the numbers of larvae recovered from both groups are shown in table I. There seems to be a correlation between the length and day of onset of hibernation and the numbers of larvae recovered from the cold room group. The wide variation in the hibernation pattern, the exceptionally low recovery in one of the warm room animals and the fact that several days of observation were missed make it difficult to draw definite conclusions from this experiment.

TABLE I. *Periods of host hibernation and numbers of larvae recovered from hamsters infected with 540 larvae.*

Cold-room group				Warm-room group	
Duration of infection in days	Day of first hibernation after infection	Number of days hibernation after infection	Numbers of larvae recovered	Duration of infection in days	Numbers of larvae recovered
56	5	15	40	30	412
56	5	4	120	33	26,600
33	5	2	350	50	6,940
53	6	9	1,310	53	3,160
33	15	1	3,550	55	6,780
58	45	5	8,360		
55	53	1	11,700		
50		0	3,330		
		Mean:	3,581	Mean:	7,320
Periods of host hibernation and numbers of larvae recovered from hamsters infected with 200 larvae					
41	6	30	0	39	1,000
41	20	19	0	39	1,560
47	9	23	0	39	5,240
47	1	39	0	39	880
47	4	31	0		
				Mean:	2,170

Experiment 2. Ten hamsters which had been held in the cold room for 27 days were infected with 200 trichina larvae each. Five hamsters were kept in the warm room, five at 5 C in the cold room. One of the animals in the warm room died on the second day of infection. The remaining animals were sacrificed between the 39th and 47th days after infection.

Table I summarizes the results of this experiment. Of special interest is the negative result with one animal which was not observed to hibernate until 20 days after infection, when some muscle larvae should already have been present.

Experiment 3. Nine hamsters which had been in the cold room for 77 days and three hamsters which had been in the warm room were infected with 200 trichina larvae each. The three which had not been in the cold were left in the warm room with three from the cold room. The remaining six from the cold room were returned to the cold. Four of the latter group hibernated within 48 hours and were removed to the warm room on the fourth day after infection. The remaining two hamsters which had been returned to the cold did not hibernate and were removed to the warm room 10 days after infection. All animals were sacrificed 30 to 35 days after infection.

The animals which remained in the warm room throughout the experiment yielded 8,600, 900, and 275 larvae. Those exposed to the cold before but not after infection yielded 16,200, 6,400, and 500 larvae. No larvae were recovered from the four hamsters which hibernated 48 to 72 hours beginning on the second day after infection.

Experiment 4. In an attempt to induce hibernation in infected hamsters later in the course of infection than occurred in experiment 3, 15 hamsters were held in

the cold room for 20 days, then infected with 200 trichina each. At the same time 13 hamsters in the warm room were infected with the same dosage to serve as controls. The two groups were left at cold-room and warm-room temperatures, respectively, for 30 days after infection.

No hibernation was observed in the cold-room group during the period of observation. The surviving animals in each group were sacrificed between the 30th and 38th day after infection. Recoveries from the eight survivors in the cold-room group averaged 39,162, ranging from 9,440 to 101,340. Recoveries from the 11 survivors of the warm-room group averaged 25,890, ranging from 3,280 to 46,190. The difference between the means was not significant, Student's *t*-test indicating a difference as great as that observed might occur by chance in at least 5 percent of similar trials.

DISCUSSION

The pattern of hibernation exhibited by hamsters, periods of 4 to 15 days of hibernation alternating with active periods of 1 to several days, made it difficult to produce equal periods of temperature reduction in different hosts. Valid conclusions can be drawn from the data presented here only when the irregularity of hibernation is clearly recognized.

Since elimination of infection occurred in one animal in which hibernation was not observed until the 20th day after infection, it is possible that the lowered host temperature may eliminate muscle as well as intestinal phases of the parasite. Additional evidence is needed on this point, since prior to entering hibernation hamsters usually undergo several extensive fluctuations in body temperature. Such fluctuations may not have been detected by the method of observation used but may have influenced the course of the infection.

The rectal temperature of the hibernating hamster, as reported in the literature and as determined in the course of these experiments, is equal to or slightly above the environmental temperature. Thus the results presented here indicate that 48 to 72 hours of host temperature reduction of 5 to 7 C will completely inhibit the development of *T. spiralis* if the reduction occurs early in the course of infection. The results also suggest that later in the infection longer periods of hibernation are required to inhibit development of the parasite.

SUMMARY

1. Hibernation may either prevent or retard development of *T. spiralis* in the golden hamster.

2. Forty-eight and 72 hours of hibernation at 5 C gave complete protection to four hamsters receiving 200 *T. spiralis* larvae when the onset of hibernation was within the first 36 hours.

3. No larvae were recovered from six hamsters hibernating for periods of 19 to 39 days following infection with 200 *T. spiralis* larvae. The first observed hibernation in this group occurred from 1 to 20 days after infection.

ACKNOWLEDGMENTS

The author wishes to acknowledge the cooperation of the President and Fellows of Middlebury College. Special thanks are due to the following Middlebury undergraduate students: Dorothy B. Covalt, Marcia Gillis, Sue Lewis, Hugh

Spitzer, John Wood, and William Milier. Without their assistance the project could not have been undertaken.

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ANNOUNCEMENT

FIFTIETH ANNIVERSARY OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON.

Saturday, October 8, 1960, the Helminthological Society of Washington observed its 50th anniversary by presenting a scientific program at the University of Maryland. The morning and afternoon sessions were attended by 260 scientists from the United States, Canada (11), Europe (5), Asia (7), and Australia (2).

The evening program, following a banquet, featured Chauncey D. Leake, President of the A.A.A.S., who presented an address entitled "Paralogue and Parasite."

Two awards were presented. One was to Miss Edna M. Burher in recognition of outstanding service to the Society for having served 25 years as Secretary-Treasurer. The other, granted by the Trustees of the Brayton Howard Ransom Memorial Fund, went to Dr. James Turner "for meritorious service to parasitology and related sciences."

RESEARCH NOTE

EXPERIMENTAL STRONGYLOIDIASIS IN SHEEP AND GOATS. V. THE EFFECT OF CERTAIN ENVIRONMENTAL CONDITIONS AND CHEMICALS ON THE INFECTIVE LARVAE OF *STRONGYLOIDES PAPILLOSUS*.

Turner (1959, Am. J. Vet. Res. **20**: 102–110) demonstrated that the intestinal threadworm, *Strongyloides papillosus*, under certain conditions, caused acute distress and some deaths in lambs and kids. During the studies, incidental observations were made on the susceptibility of the infective larvae of this parasite to different environmental conditions and to several chemicals that might be used to control or prevent infection. The individual tests herein reported used larvae in groups of 100 to 125.

Effect of desiccation. A drop of distilled water containing 3d-stage larvae in suspension was placed on each of 9 glass slides and allowed to evaporate to dryness at an air temperature of 22 C. The instant at which the water completely evaporated was determined by microscopic examination. At 2 to 15 minutes after evaporation, a drop of water was added, and the larvae were observed for 30 minutes. No larvae recovered motility after being dried 5 minutes or longer; 80 percent survived a 4-minute exposure and 78 percent survived 3 minutes. The motility of control larvae in unevaporated drops of water was unaffected.

Effect of cold. Third-stage larvae were placed on wet charcoal in small vials and were exposed, in a refrigerator, to temperatures of 5.5, 3, and −5 C. The larvae were exposed for 24 to 48 hours at each temperature. They were then observed for 1.5 hours. No larvae regained motility after exposure at −5 C. At 3 C, 78 percent survived after 24 hours, but less than half or 47 percent survived the 48-hour exposure. An interesting fact was that all larvae survived in cultures at room temperature of 24 C.

Effect of direct sunlight. Tests were made of the effect on larvae of sunlight at approximately 35 C. The larvae were pipetted into a small Petri dish with just enough tap water to cover the bottom 1 to 2 mm deep. The dish was then placed so that direct rays of the sun reached the larvae without first passing through the glass. The larvae were exposed to sunlight between 1:30 and 3:30 p. m. for 15, 20, 60, and 120 minutes. After exposure, they were immediately returned to the laboratory and held at room temperature during the 60-minute observation period. Exposure to sunlight alone (minus its heating effect) for 60 and 120 minutes was very effective in destroying larval motility and perhaps reducing infectivity for lambs and kids. Only 3 percent were motile after these exposures. About 74 percent survived a 30-minute exposure and all survived 15 minutes. Control larvae were normal after being held 20 minutes in an incubator at 37 C.

Effect of chemicals. Third-stage larvae were introduced into 95 percent and 70 percent ethyl alcohol, kerosene, and aqueous solutions containing 1 percent lysol, 1 percent thymol, and 10 percent copper sulphate. The larvae were completely inactivated when exposed in these solutions for 4, 6, 60, 5, 20, and 30 minutes, respectively. Larvae that were introduced into a 1 percent aqueous iodine solution were inactivated instantaneously. At the conclusion of this test, the solutions were highly diluted, and the larvae were observed at intervals for 30 hours to see if they would recover motility. None of the larvae became active and were presumed dead.—JAMES H. TURNER AND GRANT I. WILSON, *Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.*

INCIDENCE OF *CAPILLARIA HEPATICA* IN POPULATIONS OF THE FLORIDA DEER MOUSE, *PEROMYSCUS FLORIDANUS*

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The nematode *Capillaria hepatica* (Bancroft, 1893) has been recorded from a number of North American rodents as well as the introduced brown rat and house mouse. However, little is known of the epizootiology of this parasite in native rodent populations in this country. A survey of the parasites of the Florida deer mouse, *Peromyscus floridanus* (Chapman), in connection with a study of the life history and ecology of this endemic Florida rodent has provided data on the incidence of *C. hepatica* infections in populations in different habitats together with some information on possible age and sex differences in infection rates. *P. floridanus* represents a new host record for *C. hepatica,* which has previously been recorded in Canada from *P. maniculatus* (see Lubinsky, 1956 and Freeman, 1958). The reports of *C. hepatica* from the cotton rat, *Sigmodon hispidus,* in Texas (Read, 1949) and from the fox squirrel, *Sciurus niger,* in Louisiana (McQuown, 1954) appear to be the only other published records of this parasite in wild mammals in the southern United States.

MATERIALS AND METHODS

Specimens were collected by live trapping at 36 stations in seven countries in peninsular Florida from December 1956 through October 1959. Thirty-four of the stations were located in six counties (Alachua, Clay, Gilchrist, Levy, Putnam, and St. Johns) in the northern part of the peninsula, while two others were in Highlands County in southcentral Florida. A standard trapping procedure consisting of lines with a varying number of pairs of traps set at intervals of approximately 50 feet was used throughout the study. Trapping periods generally ranged from 1 to 3 days, and an effort was made to obtain adequate samples of mice throughout the year in each major habitat type studied. The number of animals taken per 100 trapnights is used in this paper as an index of relative densities of populations in the various habitats and localities trapped.

A total of 723 mice was examined. The specimens were assigned to two age classes. Mice in juvenile pelage or undergoing the postjuvenile molt (chronological age 4 to 16 weeks) were classed as young, and those in which the postjuvenile molt was complete were considered to be adult. The liver of each specimen was carefully examined at autopsy for the presence or absence of *C. hepatica* lesions and then preserved in 10 per cent formalin. The preserved livers of 698 of the 723 specimens examined were comminuted and subjected to the formalin-ether concentration technic of Ritchie (1948). Samples of the sediment obtained following centrifugation were examined microscopically for the presence of *C. hepatica* or other helminth eggs. In this procedure, the livers were prepared separately and the entire organ utilized. The mean number of eggs appearing in 10 randomly selected 10× fields on the slide was used as a rough index of the abundance of eggs in positive cases. No infections were found in microscopic examinations that had not been detected grossly at the time of autopsy.

The characteristic appearance of the lesions in the livers of infected mice together with the structural details and size (mean, 60 by 35 microns) of the contained ova were the principal criteria used in the identification of the parasite involved as *C. hepatica,* since specimens of the adult worms were difficult to remove intact from the liver tissue and in many cases were not apparent on examination.

From 1 to 16 examples of each of the known major habitats of *P. floridanus* were sampled,

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the number of stations of each habitat type included in the study bearing a general relationship to the extensiveness and suitability of the habitat for *P. floridanus*. These habitat types include sand pine scrub, longleaf pine/turkey oak, Caribbean pine/turkey oak, longleaf pine flatwoods, and ecotonal situations. Included in the latter classification are transition zones between flatwoods or longleaf pine/turkey oak and mesic hammocks in addition to live oak and upland hammocks. These plant associations have previously been described by Rogers (1933), Carr (1940), Laessle (1942, 1958), and others.

Sand pine scrub, longleaf pine/turkey oak, and Caribbean pine/turkey oak associations occur on very sandy well-drained soils and are typically xeromorphic. Of six wooded habitats in northern Florida studied by Rogers (1933), sand pine scrub and longleaf pine/turkey oak had the highest over-all evaporation rates and the greatest evaporation increments due to insolation. The Caribbean pine/turkey oak association is rather intermediate in vegetative aspect between scrub and longleaf pine/turkey oak (Laessle, 1958), and general observations indicate that it is quite similar to these in microclimatic characteristics as well.

Pine flatwoods and ecotonal associations are moister than the foregoing habitats. Flatwoods occupy areas of level topography and poor drainage, and though they may be quite dry during certain seasons or years their soils quickly become saturated during rainy periods and extensive areas of standing water may be present. Ecotonal situations appear to have comparatively more stable microclimates than flatwoods and in general tend toward more truly mesophytic conditions.

RESULTS

Mice with *C. hepatica* infections were collected at two stations in Highlands County, two in Levy County, and three in St. Johns County. Twenty-one (2.9 percent) of the total of 723 specimens examined were infected, while 12.7 percent of the mice from infected populations carried *C. hepatica*.

The majority of infected mice had the entire liver affected, the organ usually being considerably discolored from the concentrations of eggs and often much swollen and lobulated. Such individuals did not appear to be in noticeably poorer physical condition than uninfected animals as judged by general appearance, behavior, and body weight. Infected females included animals at various stages of pregnancy or lactating, and males carrying *C. hepatica* which were taken during the breeding season appeared to be fully fertile, having well developed testes and accessories and containing quantities of spermatozoa in the cauda epididymis. Thus, there was no obvious evidence of interference with normal reproductive functions by *C. hepatica* infections. No direct or presumptive evidence was obtained of any mortality in *P. floridanus* that could be traced directly or indirectly to *C. hepatica* infections.

The incidence of infections and relative population densities in each of the habitats trapped are given in table 1. These data show that of the five habitat

TABLE 1. *Habitats sampled and incidence of* C. hepatica *infections in* P. floridanus.

Habitat type	Number of stations trapped	Stations with infections	Relative density, mean and range	Number of specimens examined	Number infected	Percentage infected
Sand pine scrub	8	6	13.2 (1.2 – 36.1)	120	18*	15.0
Caribbean pine/Turkey oak	1	1	18.1 (2.6, 21.0)	45	3	6.7
Longleaf pine/Turkey oak	16	0	3.5 (0.2 – 10.0)	246	0	0
Longleaf pine flatwoods	2	0	6.4 (5.2, 6.9)	56	0	0
Ecotonal	9	0	7.4 (1.3 – 25.0)	256	0	0
All habitats	36	7	6.4 (0.2 – 36.1)	723	21	2.9

* Includes one specimen from mesic hammock near scrub habitat.

types investigated *C. hepatica* infections were restricted to populations of *P. floridanus* inhabiting sand pine scrub and Caribbean pine/turkey oak associations. The single infection found outside of these habitats was in a mouse collected in a mesic

hammock a short distance from an adjoining scrub, and it is probable that the animal was in actuality a scrub resident that had chanced to wander into the hammock. Since only one Caribbean pine/turkey oak station was trapped, the actual status of *C. hepatica* infections in this habitat type, which is of limited extent and distribution in the state, is questionable. Furthermore, the example studied was located in close proximity to a typical scrub in which *C. hepatica* was present, and this may have been a factor in the presence of infections. The difference in the proportion of infected mice in the combined scrub samples and the Caribbean pine/turkey oak group was not statistically significant ($X^2 = 1.99$, $P > .05$).

Although the individual samples are small, the numbers of infected and non-infected mice from different stations within the habitat types where *C. hepatica* occurred suggests appreciable variation in infection rates. In three sand pine scrub stations trapped once in 1957 or 1959 and having densities ranging from 1.4 to 7.6 mice per 100 trapnights, none of six mice collected was infected. At three other scrub stations with population indices of 1.2, 3.5, and 3.6 the single specimen taken at each was positive for *C. hepatica,* perhaps indicating a rather high infection rate despite the apparently low numbers. Two similar scrub habitats located within 3 miles of one another had population densities of 16.8 and 3.6 when trapped in 1957. In the first, none of 42 adults examined was infected, whereas in the other all of 4 adults collected were positive for *C. hepatica.*

Some information on variation in infection rates from year to year is available for the Caribbean pine/turkey oak station and three scrub populations trapped for 2 or 3 years between 1956 and 1959. In the former 2 of 12 adult mice were infected in 1956 and 1 of 18 in 1957. No adults were taken in 1959. Population indices for these years were 10.8, 21.0, and 2.6, respectively. One scrub population had consistently high populations in 1957, 1958, and 1959 (16.8, 22.0, and 26.7) yet only one specimen, a juvenile, was infected out of a total of 78 examined during this interval. At a nearby station in the same habitat, a density of 3.6 was recorded in 1957 and 31.8 in 1959. In both years every adult (7) examined was infected with *C. hepatica.* A third scrub station had a population density of 36.1 in 1957 when 6 of 16 adult mice taken were infected. In 1959 the density had declined to 2.6, although the only adult collected had *C. hepatica,* which suggests that the parasite might still have been frequent in the population.

Data on the incidence of *C. hepatica* in different age and sex classes of mice from infected populations are presented in table 2. These figures suggest the pos-

TABLE 2. *Incidence of* C. hepatica *in different age and sex classes of* P. floridanus *from infected populations.*

	Adults			Young			Combined total
	Males	Females	Total	Males	Females	Total	
Number examined	59	70	129	21	15	36	165
Number infected	11	9	20	—	1	1	21*
Percent infected	18.6	12.8	15.5	—	6.7	2.8	12.7

* Includes one specimen from hammock near scrub habitat.

sibility of a higher infection rate in adults as compared to young and in adult males as compared to adult females. The observed difference between infections in adults and young is statistically significant ($X^2 = 4.11$, $P < .05$), whereas that between adult males and females is not ($X^2 = 0.79$, $P > .05$).

Infections were recorded in February, April, May, June, July, August, October, and November. No seasonal differences in infection rates such as reported by Freeman and Wright (1960) for *P. maniculatus* are apparent, although the data are obviously too few to be conclusive.

DISCUSSION

Information on the extent of *C. hepatica* infections in wild populations of brown rats, house mice, and several species of native North American rodents has been given by Ameel (1942), Freeman and Wright (1960), Harkema (1936), Herman (1939), Lubinsky (1957), Luttermoser (1936a, 1938), McQuown (1954), Meyer and Reilly (1950), Penn (1942), Price and Chitwood (1931), and Shorb (1931). Compared to the values reported in these studies, the over-all incidence of *C. hepatica* infections in *P. floridanus* is at a relatively low level, although the rate of infections in some populations may be quite high.

There is little evidence as to the actual effects of *C. hepatica* infections on mortality rates or other attributes of wild rodent populations in which the parasite occurs. Luttermoser (1936a) and Herman (1939) noted that many of the infections occurring in wild rats were of low intensity, and experimental evidence indicates that this parasite has a more injurious effect on the mouse than the rat, the latter being considered as the more suitable host (Luttermoser, 1936b; 1938). Although many of the infections observed in *P. floridanus* would probably be classed as heavy according to the criterion of Herman (1939), individuals carrying such infections showed no sign of physical inpairment, nor was any direct or indirect evidence of mortality that could be attributed to *C. hepatica* infection obtained during the course of the study. It seems probable, therefore, that *C. hepatica* infections do not constitute an important source of direct mortality in *P. floridanus* populations.

Our data further indicate a further striking restriction of *C. hepatica* infections to only two of the five major habitat types studied, namely, sand pine scrub and Caribbean pine/turkey oak. If the parasite is present in mice in other habitats its incidence must be very low indeed. Sand pine scrub and Caribbean pine/turkey oak are both typically xeric associations and in this study had the highest average mouse population densities of any of the habitats investigated.

In view of the cosmopolitan distribution and broad host tolerance of *C. hepatica* it appears unlikely that the range of environmental factors encountered in the Florida habitats studied would in themselves be limiting in any case, although the possibility that some specific critical condition of soil or some other physical environmental factor may be involved cannot be excluded. It would seem particularly difficult to reconcile a high infection rate in extremely sandy, well-drained soils with the equal or higher rates that have been reported previously in the United States for the muskrat, *Ondatra zibethicus,* which of course occupies aquatic situations. Further evidence against habitat conditions per se as being a principal factor in determining the pattern of infections observed is provided by the relation between infections and environmental conditions between the major habitat types studied, particularly in the case of sand pine scrub or Caribbean pine/turkey oak and longleaf pine/turkey oak associations. On the basis of general field observations, at least gross environmental conditions in these three habitats overlapped in many instances. Thus a number of longleaf pine/turkey oak stations were ap-

parently fully as xeric as some of the scrub or the Caribbean pine/turkey oak stations from which infected mice were obtained, yet all of the former habitats were negative for *C. hepatica.*

Freeman and Wright (1960) noted habitat differences in the percentage of infections in *P. maniculatus* but were inclined to consider the differences as being correlated directly with population density rather than the habitats themselves. Our data do not show such a clear-cut correlation between population level and incidence of *C. hepatica.* Although the over-all average density of scrub and Caribbean pine/turkey oak mouse populations is higher than in the other habitats studied, this relationship does not hold when comparisons are made between stations in infected and uninfected habitat types of grossly similar physical environments or between infected scrub stations. A number of stations in other habitat classes, particularly longleaf pine/turkey oak, with at least apparently similar environments had higher populations than the Caribbean pine/turkey oak or scrubs in which infections occurred. Furthermore, there was no clear correlation between the presence or frequency of infections and density levels within scrub habitats. The present data also suggest the possibility that *C. hepatica* may persist in a given population, perhaps even maintaining about the same infection rate, despite the occurrence of marked fluctuations in host numbers.

It appears, therefore, that neither population density nor general physical environmental conditions are adequate to account for the narrow ecologic restriction of *C. hepatica* in *P. floridanus* populations and that other factors must be implicated. Variation in the extent of predation and cannibalism in different habitat types is undoubtedly important, since these are probably the chief egg releasing mechanisms. Habitat differences in the kinds, abundance, and intensity of utilization of nesting sites, which might serve as foci of infections as postulated for a population of *P. maniculatus* by Freeman and Wright (1960), may also exist. Such factors as the types and availability of foods in different habitats, the incidence of infections in small mammal associates, the extensiveness and relative isolation of habitats, and the spatial relationships and mobility of the mouse populations might also be involved, as well as possible genetic differences in susceptibility to infection of mice in different and relatively isolated habitat types. However, on the basis of the data now available on the ecology and behavior of *P. floridanus* populations in different habitat types, no condition related to one or more of the above factors and which is exclusive to scrub and Caribbean pine/turkey oak habitats is apparent. Therefore, no satisfactory explanation for the observed habitat restriction of *C. hepatica* infections found in this study can presently be offered.

It is of interest to note that eggs of another helminth, an as yet unidentified dicrocoeliid, were also obtained from *P. floridanus* livers and a similarly strong habitat association found. In this case, however, the parasite was recorded only from the moister habitats. The explanation seems relatively simple, the occurrence of the parasite in the mice probably being correlated with the ecological distribution of an intermediate molluscan host.

Herman (1939) found only 30.5 percent of young rats infected with *C. hepatica* as compared to 91.5 percent of the adults. A similar age difference in infection rates has also been found for *P. maniculatus* (Freeman and Wright, 1960) and appears to hold for *P. floridanus* also. The apparently lower infection rates in young animals may be explained on the basis that they have had a shorter period of time

in which to contract an infection and may also have smaller home ranges than adults, which would tend to lessen the probability of exposure. An alternative interpretation might be that infections cause a higher mortality in young than adults, infected young thus disappearing more rapidly from the population and having a lower probability of capture. We believe that even if this were the case a higher percentage of young with at least early infections would still be expected in the samples. Freeman and Wright (1960) would apparently attribute the low incidence of *C. hepatica* infections in young *P. maniculatus* in Algonquin Park to the fact that infections are primarily acquired in winter nests in this area, but we can see no parallel situation in our data.

Although not statistically significant, the difference in the proportion of infected adult males and females suggests the possibility of a higher infection rate in the former. If true, this might reflect a difference in the activity range of the sexes, the males having an average larger home range and consequently a greater probability of exposure to infective eggs.

ACKNOWLEDGMENTS

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SUMMARY

The incidence of *C. hepatica* infections in a total of 723 Florida deer mice, *P. floridanus,* collected from a number of localities and five major habitat types was 2.9 percent. Considering only those populations from which infected mice were collected, the incidence of infection was 12.7 percent. Infections occurred in only two, sand pine scrub and Caribbean pine/turkey oak, of the five habitats sampled. A significantly higher proportion of adults than young was infected, and a higher infection rate in adult males than females is also suggested, although the difference is not statistically significant.

Physical environmental features per se and host population levels do not seem adequate to explain the restricted ecologic distribution of the parasite, and it is suggested that one or more additional factors relating to particular aspects of the population ecology or behavior of the mice in particular habitats must be more strongly involved. Available data, however, do not permit further analysis. The difference in adult-young infection rate is suggested as being due to the shorter time available for infection in the young and their possibly more limited range of movements, which would tend to reduce the probability of exposure. The latter explanation may also apply to a possible difference in infection rate between adult males and females.

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RESEARCH NOTE

MICROTETRAMERES IN THE BRONZED GRACKLE AND THE EASTERN MEADOWLARK.

Two apparently new host records have been established for the nematode genus *Microtetrameres*. Cram (1927, U. S. Nat. Mus. Bull. No. 140) lists this genus as being found in the crow of North America. Boyd (1951, J. Parasit. **37:** 76) reported what she identified tentatively as the larvae of *Microtetrameres helix* in a starling. Schell (1953, Tr. Am. Micr. Soc. **72:** 227) reported four new species of this genus (*Microtetrameres bubo, M. aquila, M. accipiter, M. corax*) in western United States from the great horned owl, goshawk, golden eagle, and raven. Boyd (1956, Proc. Helm. Soc. **23:** 70) found a new species, *Microtetrameres spiculata*, in the New England blue jay. Mawson (1956, Can. J. Zool. **34:** 193) extended the list of hosts by reporting a new species, *Microtetrameres canadensis*, from the Canadian snowy owl and great blue heron. The site in all these reported hosts was the proventriculus.

During some research in avian helminthology, one female *Microtetrameres* sp. was found in the bronzed grackle, *Quiscalus versicolor*, and two females of the same genus were found in the eastern meadowlark, *Sturnella magna*. No males were recovered. The nematodes were found in the proventriculus of the meadowlark and in the visceral washings of the bronzed grackle. One of 3 meadowlarks and 1 of 13 grackles examined were infected. These hosts seem to be news records. They were collected in Chickasaw County, Iowa.

Grackles have been reported previously as hosts for other members of the nematode family Spiruridae. *Tetrameres* sp. has been reported from *Quiscalus quiscalus aglaeus* by Wehr (1934, Proc. Helm. Soc. Wash. **1:** 50).—CHARLES J. ELLIS, *Department of Science, Iowa State Teachers College, Cedar Falls, Iowa.*

LIFE HISTORY STUDIES ON *PORROCAECUM ENSICAUDATUM* (NEMATODA), AN AVIAN NEMATODE. I. EXPERIMENTAL OBSERVATIONS IN THE CHICKEN*

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Members of the genus *Porrocaecum* have been known for more than a century and a half, yet life history studies are almost non-existent. Only recently have Schiller (1954) and Scott (1953, 1954, 1955) reported on the life cycle of *P. decipiens*, a parasite of various fish-eating mammals, and Osche (1955) on *P. talpae*, a parasite of carnivorous birds. Mawson (1956) reported data on *P. ensicaudatum*, from terrestrial birds, and Mozgovoi (1952) on *P. crassum*, a parasite of aquatic birds.

The present work is an attempt to add to knowledge of life histories in this genus. Studies involved experimental investigation of the life cycle of *P. ensicaudatum*, an intestinal parasite of robins and other ground-feeding birds. Because of certain difficulties encountered in the capture and maintainance of live robins and in obtaining the large numbers required for sacrifice, experiments were conducted in an effort to determine if 24-hour-old chickens could be substituted for natural hosts. It was hoped that the potential economic importance of this parasite could also be ascertained by these infections.

MATERIALS AND METHODS

Intact earthworms were collected at night on the University of Illinois campus. Worms were stored at 7 to 10 C in tins lined with slightly moistened paper toweling and filled with soil and vegetation from the collection area. Toweling was changed frequently since excess moisture caused worms to die.

The earthworm's gut was cleansed prior to sectioning. Worms were placed in covered containers packed with slightly dampened paper toweling and kept there until their droppings were free of soil. They were anesthetized in water to which 70 percent ethanol was added a few drops at a time, were washed clean of external debris and mucus and placed in 10 percent alcohol for about one and one-half hours, or until they no longer responded to pinching. Worms were then fixed in Bouin's fluid and routinely prepared for sectioning and staining. Sections were cut at 10 microns, and were stained with Erlich's acid hematoxylin and counterstained with 0.5 percent solution of eosin in 95 percent alcohol, or with Mallory's triple stain.

Immobilized earthworms were identified according to the keys of Eddy and Hodson (1947), Olson (1928) and Smith (1928). Three keys were used simultaneously in order to verify the identifications.

Specimens of *Porrocaecum* were fixed in A.F.A. or Looss' fixative (2 percent solution of glycerine in 70 percent ethanol). Worms were cleared in glycerine prior to being measured. The larger worms were measured by projection with the Leitz Edinger apparatus; the smaller ones were measured by means of a calibrated micrometer, or by camera lucida projection.

Birds were infected by several methods, including feeding with infected earthworms. This feeding method was abandoned because it was not possible to control the larvae fed to each chicken, and because earthworms carry other helminths, such as *Syngamus trachea*, which might injure the birds. The method adopted was to remove larvae from earthworms and to introduce them, in 0.85 percent saline, with a medicine dropper into the esophagus of the chick.

All recorded measurements are in millimeters unless otherwise indicated.

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* Based on a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Zoology in the Graduate College of the University of Illinois.

THE INFECTIVE LARVA

Infective larvae of *Porrocaecum ensicaudatum* were isolated from the ventral blood vessel and hearts of the earthworm, *Lumbricus terrestris* (fig. 1). Larvae

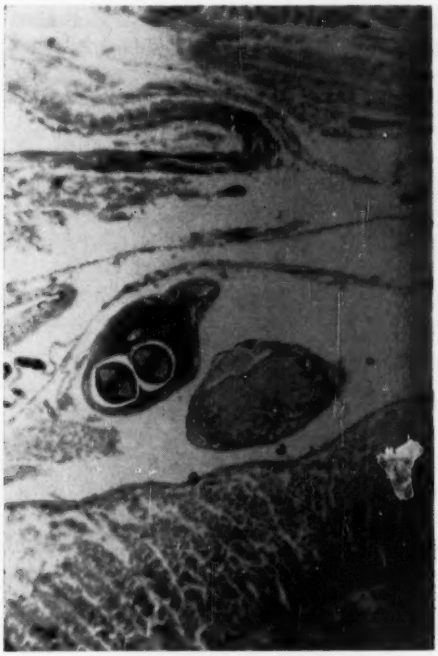

FIGURE 1. Cross section of an earthworm showing infective larvae in the ventral blood vessel.

were usually situated anterior to the clitellum and were not observed in the dorsal blood vessel.

The larva has a transparent body wall and a dark brown, almost black, intestine. The esophagus is clearly distinguishable as the *Porrocaecum* type, i.e., exhibiting a long anterior muscular part, followed by a short, oblong and wider ventriculus. Also present is a small intestinal diverticulum or caecum, which is directed anteriorly over the postero-dorsal surface of the ventriculus (plate I, figs. 1, 2).

The anterior end of the larva tapers very slightly and terminates rather bluntly. The typical ascarid lip arrangement is not yet present. The posterior end tapers, becoming more acute behind the anus, and terminates in a blunt, conically shaped tail (plate I, fig. 9). A nerve ring is visible surrounding the middle of the muscular portion of the esophagus. The excretory pore opens a short distance posterior to the nerve ring. A cuticular boring tooth is not apparent. The larva is surrounded by a sheath which is difficult to see except at the ends. This sheath forms a prepuce-like structure around the anterior end (plate I, fig. 2). The genital anlage is observed just posterior to the middle of the body as an ovoid mass ventral to the overarching intestine (plate I, fig. 1).

The brief description by Osche (1955) did not mention the genital anlage, the

excretory pore, or the absence of the cuticular boring tooth. Osche's measurements agree generally with the following, which were obtained for the infective larvae used in this study. Infective larvae measured 4.03 to 4.82 long and 0.112 to 0.139 wide. The esophagus was 0.490 to 0.602 long; ventriculus 0.112 to 0.140 long; caecum 0.017 to 0.037 long; tail 0.122 to 0.156 long. The distance from anterior end to nerve ring was 0.196 to 0.244 and to the genital anlage 2.21 to 2.53.

OBSERVATIONS AND RESULTS

In these experiments two breeds of chickens were used: White Leghorns from a local commercial hatchery, and a breed from the University of Illinois Poultry Department. The second strain was the result of a cross between a New Hampshire male and a Columbia female; the latter was the F_2 of a cross between a Plymouth Rock and a Rhode Island Red. Infection was more easily achieved in the University stock than the White Leghorns; therefore, this was the breed employed in the following work. Birds, approximately 24 hours old, were given varying doses of infective larvae and sacrificed at time intervals ranging from 1 hour to 45 days.

1 to 48 hours. Within an hour after inoculation, larvae were embedded in the esophagus, proventriculus and anterior portion of the gizzard. No morphological changes occurred for the first 36 hours, when evidence of molting was first observed. Sheaths of larvae removed from the host could be seen projecting from the posterior ends of several worms (plate I, fig. 10); in other specimens the sheath, with the esophageal lining, extended forward for a short distance (plate I, fig. 3). After 48 hours, all the larvae had molted and were located in the gizzard, under the yellow, acellular gizzard lining. As the lining was peeled off, the helminths adhered to it, indicating they had not penetrated into the muscle. At this time the genital anlage, in 2 specimens, had begun to enlarge. Although there was no definite vulval opening or vaginal tube, faint lines perpendicular to the longitudinal axis of the body marked the future site of these structures. Three incompletely formed lips could be distinguished at the anterior end (plate I, fig. 4).

3 to 5 days. Worms were recovered from the duodenal mucosa which revealed small hemorrhagic spots around the larvae. During the 3d day the genital anlage had started to bend in an antero-ventral direction, at the same time lengthening and bifurcating toward the posterior end (plate I, fig. 15). By the 5th day females could definitely be distinguished by a vulva, a well defined vagina and a pair of tubes representing uteri, oviducts, and ovaries (plate I, fig. 16). Males showed rudiments of testes as short tubes originating in the region of the genital anlage, coursing caudally in the same manner as the primordia of the female system. Formation of the spicule apparatus was noted as a crescent-shaped band of tissue, dorsal to the rectum and posterior to the large rectal gland. The intestine and esophagus had increased in length as evidenced by their bending (plate I, fig. 8).

10 to 14 days. On the 10th day preparations for a 2d molt were indicated by the presence of a double cuticle. At this time the spicule pouch had opened into the cloaca (plate I, fig. 12). Worms of two different sizes were recovered on the 14th day, one group noticeably longer and farther down the intestine. The body wall of the longer worms was creamy-white and somewhat opaque, in contrast to the more transparent body wall of the shorter forms. The less advanced worms retained the immature lip pattern and double cuticle. The longer larvae had molted and showed

a labial pattern with dentigerous ridges typical of adult ascarids (plate I, fig. 5). Copulatory muscles and well developed spicules were evident in the males. The female reproductive system was fully developed with the oviducts beginning to recurve anteriorly.

18 to 21 days. Nodules were present on the outer intestinal wall of the chicken. Those closest to the gizzard were quite small and showed the black thread-like remains of earlier larval stages. Nodules farther down the intestine were traversed by thick yellow-tan ridges resembling the coloration of the older worms' intestine. Some of the nematodes were free in the intestinal lumen; others were still embedded in the tissue. The cuticle was opalescent; spicules could be seen as dark brown, slightly curved rods, situated dorsally to the cloaca; tails of the males were slightly recurved in a ventral direction; copulatory muscles were well developed (plate I, fig. 13). Oviducts of the females were long, extensively coiled, recurved and extended a short distance anterior to the vagina (plate I, fig. 17).

25 to 45 days. Helminths recovered from the 25-day infection were similar in appearance to those discussed from the 18 to 21 day infections. A single, dead, female nematode, resembling those of the 14-day post-molt infection, was removed from the intestinal tissue of a 28-day infection, after which time no worms were found. In some birds the only evidence of infection was the presence of nodules on the intestinal wall. Generally, the nodules conformed to the previous descriptions, although after 35 days some were manifested as whitish iridescent patches.

Male helminths recovered after the first molt within the chicken had the following dimensions: length 4.96 to 9.76; width 0.122 to 0.292; length of esophagus (including ventriculus) 0.588 to 1.022; ventriculus 0.112 to 0.210 long; caecum 0.034 to 0.143; tail length 0.129 to 0.194; spicule length 0.129; and distance from the anterior end to the nerve ring 0.238 to 0.336. The measurements of males recovered after the second molt were: length 12.37 to 24.75; width 0.265 to 0.504; length of esophagus (including ventriculus) 1.160 to 1.839; ventriculus 0.210 to 0.285 long; caecum 0.041 to 0.182; tail length 0.177 to 0.266; spicule length 0.145 to 0.575; and distance from anterior end to nerve ring 0.308 to 0.560.

Measurements for females after the first molt were: length 4.93 to 10.83; width 0.119 to 0.266; length of esophagus (including ventriculus) 0.574 to 1.148; ventriculus 0.126 to 0.210; caecum 0.034 to 0.095; tail length 0.139 to 0.238; distance from anterior end to vulva 2.46 to 5.37; and distance to nerve ring 0.224 to 0.350. After the second molt, the females ranged in size as follows: length 10.90 to 33.70; width 0.214 to 0.560; length of esophagus (including ventriculus) 1.148 to 3.797; ventriculus 0.224 to 0.448; caecum 0.058 to 0.136; distance from anterior end to vulva 5.93 to 15.93; and distance to the nerve ring 0.308 to 0.578.

DISCUSSION

The life cycle of *Porrocaecum ensicaudatum* involves two hosts, a terrestrial oligochaete and a bird. In contrast, Osche (1955) demonstrated that *P. talpae* utilizes a small insectivorous mammal as the intermediate host and a carnivorous bird as the final host. Mozgovoi (1952) determined the intermediate host of *P. crassum* to be an earthworm and the definitive host an aquatic bird. Schiller (1954) and Scott (1953, 1954) showed that *P. decipiens* uses a marine fish for the intermediate host and a marine mammal as the final host. Schiller's work was not experimental, but he stated that the parasitic form in the fish was morphologically identical

with the younger forms found in the sea otter, *Enhydra lutris*. Scott proved that these helminths were larvae of this species by feeding worms from the fish to the harbor seal, *Phoca vitulina*, and by recovering the adults. As with Schiller's study, the information concerning *P. ensicaudatum* by Mawson (1956) and Osche (1958) was not based on experimental findings, but rather on descriptions of three different stages recovered from ground-feeding birds. Mawson referred to her material as groups 1, 2, and 3, while Osche designated his material as stages III, IV, and V. These stages correspond with the infective larva and the first and second molts respectively within the chicken. Mawson (1956) stated that sexual differentiation does not take place in group 2, but in group 3. Experimental data in the present work shows that differentiation of the sexes does occur in group 2. Females can be determined quite early, as faint indications of the vaginal site could be observed 48 hours after molting of the infective larva and by 72 hours a vagina was developed (plate I, fig. 15). First indications of the spicule apparatus were noted on the 5th day.

Data of Scott (1955) and Mozgovoi (1952) showed that the first molt of *Porrocaecum decipiens* and *P. crassum* and development to the infective larva must occur in an intermediate host. Information concerning the fate of the ova of *P. talpae* has not been reported. Although females were well developed by the 18th day, no ova were recovered from experimental infections of *P. ensicaudatum*. Eggs were not discernible in the oviducts even in 25-day infections. A reason for the absence of ova may be due to the fact that the chicken is not the natural host. Preliminary studies of ova collected from helminths in wild birds indicate that some embryonation occurs outside of the host. As the studies are incomplete, subsequent development can only be surmised. Probably the first molt occurs within the egg as in most ascarids. It may be assumed that the 1st-stage larvae emerge in the intestine of the intermediate host which, in the case of *P. ensicaudatum*, is the earthworm. In this species subsequent development is characterized by further growth, another molt, and migration to the ventral blood vessel. These events can only be proved by further investigations.

The low number of recovered luminal forms probably can be partially explained by the fact that some of the infective larvae did not penetrate the tissue of the gizzard. Since many worms passed through this organ and apparently did not penetrate the intestinal tissue, they were subsequently shed with the feces. Histological examination of the intestinal nodules showed the presence of degenerating nematodes. This host reaction to the tissue phase is another contributory factor to the low recovery of luminal forms.

The degree of development and growth of *P. ensicaudatum* in experimental chickens suggests that these birds may be potential hosts; however, the economic importance appears to be negligible, for there were no deaths among the fowls directly attributed to the parasite. Growth, general appearance, behavior, and feeding of the infected birds were no different from that in uninfected animals. Neither diarrhea nor bloody stools were observed. The nodules, which appeared on the intestinal wall at the time of tissue penetration by the parasite, eventually disappeared. Therefore, evidence indicates that, although *P. ensicaudatum* will undergo development in the chicken, little harm is manifested.

The helminths in these experiments agree with other descriptions of *Porrocaecum ensicaudatum*. The labial pulp possessed the lateral wings characteristic of *P.*

ensicaudatum (plate I, fig. 7), but which are lacking in *P. brevispiculum* Webster (1943). Although Mozgovoi found that earthworms could serve as the intermediate host for *P. crassum,* Levin (1957) does not believe that the larvae used in his study are co-specific with *P. crassum* for two reasons: (1) Morphological differences such as the greater length of the intestinal caecum of *P. crassum* and (2) failure of the larvae discussed in this paper to infect 24-hour-old ducklings.

As a result of this study, the author concurs with Osche (1955) and Mawson (1956) that there is an error in the identification of the nematode designated as *Spiroptera turdi.* According to von Linstow (1884), *S. turdi* was first reported by Molin in 1860 as a larval form which occurred in the *"Magenhaut"* of *Turdus sp.* In 1877, von Linstow found a larval nematode under the stomach membranes of *T. iliacus,* which he named *Filaria turdi.* Seven years later he recovered what was thought to be the adult male of this species in the stomach tissues of *T. pilaris.* At the same time *F. turdi* was shown to be the same as *S. turdi.* Von Linstow (1887) discovered this parasite in *T. merula* and also reported having previously seen it in the intestine of *Sturnus vulgaris* and *Crocidura sp.* At this time he thought the intermediate host to be an insect. Cori (1898) described a larval nematode from the ventral blood vessel of *Lumbricus terrestris* which proved to be morphologically identical with larval forms of *Spiroptera turdi* found in the stomachs of birds. Although Cori performed no experimental feedings, he correctly stated that the earthworm was the intermediate host; however, a comparison of the infective larva of *P. ensicaudatum* from the earthworm with that of *S. turdi* shows that these species are identical. A comparison of the 4th-stage larvae of *P. ensicaudatum* with the adult worms of von Linstow shows that these are also the same species. Thus experimental studies on the life history of *P. ensicaudatum* have demonstrated that *S. turdi* is not a valid species, but represents a name given to the larval stages of the former species. Correct designation for this helminth should be *Porrocaecum ensicaudatum,* which was named *Ascaris ensicaudata* by Rudolphi (1809). *A. ensicaudata* was so listed by various authors, including von Linstow (1875, 1884), until Raillet and Henry erected the genus *Porrocaecum* in 1912.

SUMMARY

Certain facts in the life history of *P. ensicaudatum* are revealed by experimental infections in chickens. The infective larvae are located in the ventral blood vessel and hearts of *Lumbricus terrestris.* Within an hour after ingestion by the definitive host, the parasite begins to penetrate the horny layer of the gizzard. Exsheathing of the infective larvae is accomplished by the 48th hour and occurs when the larvae are situated between the horny layer and the muscle wall of the gizzard. By the 3d day the parasite is found in the duodenal wall; the female reproductive system has started to differentiate. Rudiments of testes are noted on the 5th day. The spicule pouch opens into the cloaca by the 10th day when preparation for the second molt is observed. Completion of ecdysis occurs generally on the 14th day. Emergence from the intestinal tissue to the lumen begins on the 18th day with subsequent growth. Although smaller, the worm resembles the adult at this stage.

These studies have confirmed that *Spiroptera turdi* Molin is the larval form of *Porrocaecum ensicaudatum.*

ACKNOWLEDGMENTS

The author desires to express his deepest appreciation to Dr. L. J. Thomas, under whose supervision this work was completed. Thanks are also due to Dr. H. M. Scott of the College of Agriculture for his kind donation of chickens.

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EXPLANATION OF PLATE

All figures were drawn with the aid of a camera lucida.

FIGURE 1. Infective larva of *Porrocaecum ensicaudatum*.
FIGURE 2. Lateral view of anterior end of infective larva.
FIGURE 3. Anterior end of infective larva in process of molting.
FIGURE 4. Anterior end of larva after loss of infective-larval sheath.
FIGURE 5. Anterior end of *Porrocaecum ensicaudatum* from 14-day infection.
FIGURE 6. Lateral view of anterior end of adult female.
FIGURE 7. Dorsal lip of adult.
FIGURE 8. Anterior end of larva from 5-day infection showing sigmoid shape of muscular esophagus and undulating at end of intestine.
FIGURE 9. Lateral view of tail of infective larva.
FIGURE 10. Lateral view of tail of infective larva in process of molting.

FIGURE 11. Lateral view of tail of larva after loss of infective-larval sheath.
FIGURE 12. Lateral view of male from 10-day infection showing spicule pouch opening into cloaca.
FIGURE 13. Lateral view of posterior end of young male showing copulatory muscles and spicule.
FIGURE 14. Lateral view of tail of adult female showing rectal muscles.
FIGURE 15. Lateral view of vulval region from 3-day infection.
FIGURE 16. Lateral view of developing female reproductive system from 5-day infection.
FIGURE 17. Lateral view of vulval region of young adult female.

PRELIMINARY ANNOUNCEMENT OF THE THIRTY-SIXTH ANNUAL MEETING

August 27 to 31, 1961, Purdue University, Lafayette, Indiana

The thirty-sixth annual meeting of the American Society of Parasitologists will be in conjunction with the annual meeting of the American Institute of Biological Sciences.

Inquiries concerning the general plans for the meeting may be addressed to the Secretary, Dr. Francis J. Kruidenier (Department of Zoology, University of Illinois, Urbana, Illinois). Dr. S. M. Gaafar (School of Veterinary Science and Medicine, Department of Veterinary Microbiology, Purdue University, Lafayette, Indiana) has agreed to serve as Chairman of the Society's Local Committee; the other members of the Committee are Drs. John H. Greve and A. F. West.

THE HENRY BALDWIN WARD MEDAL FOR 1961

Nominations of recipients for the Henry Baldwin Ward Medal to be awarded at the next annual meeting of the Society (Purdue University, Lafayette, Indiana, August 27 to 31, 1961) should be in the hands of the Secretary not later than the first of May. They may also be sent directly to the Chairman of the Awards Committee, Dr. N. D. Levine, School of Veterinary Medicine, University of Illinois, Urbana, Illinois.

Nominating letters should state fully the qualifications of the nominee and include a biographical sketch, honors, publications, and a brief summary of major contributions.

The medal is named in honor of the Society's first president and founder of the Journal of Parasitology. It is sponsored by Parke, Davis and Company. An award of $1,000 accompanies the medal. The conditions of the award, established by the Society, are as follows:

The recipient shall be a member of the Society who by 15 years or less of self-directed investigations will have attained a position of leadership in some phase or phases of parasitological research, the term parasitology being interpreted as including in general those fields represented by reports published in the official organ of the Society, The Journal of Parasitology.

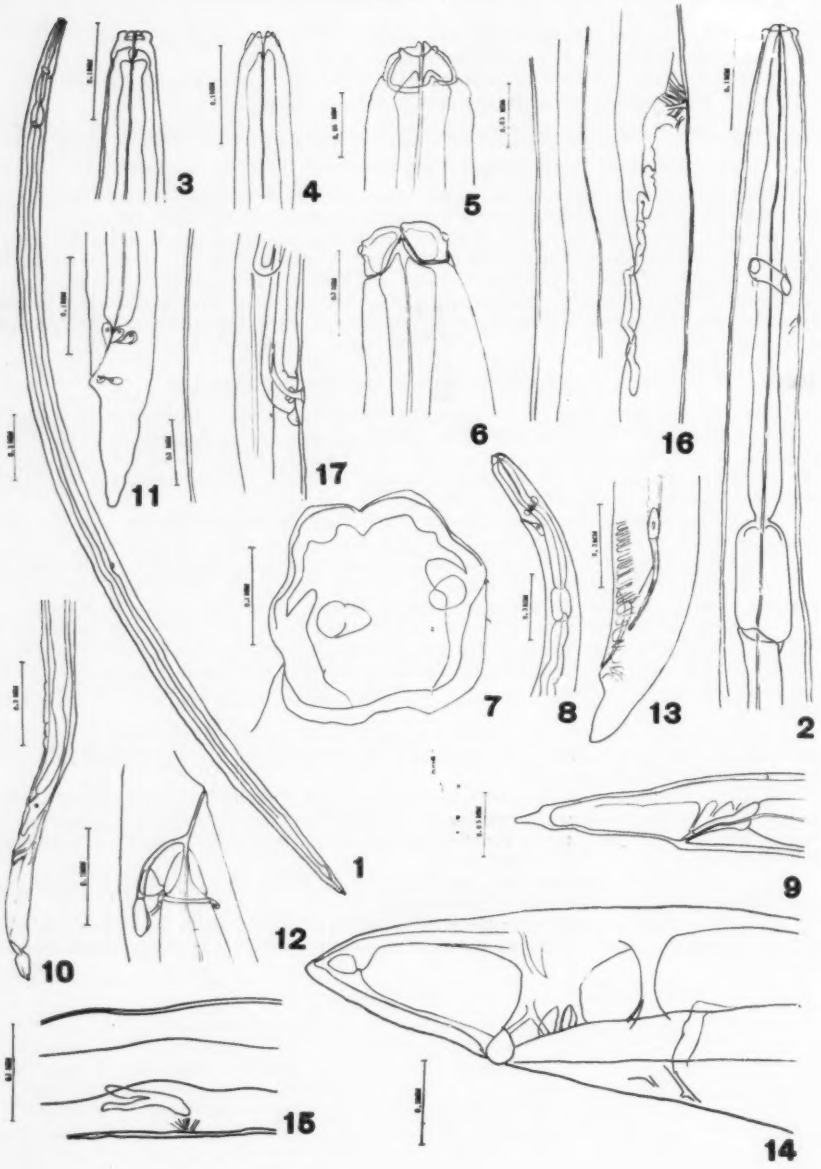
Nominations of recipients shall be made by members of the Society through the Secretary and must be received 6 months prior to the next scheduled annual meeting at which time the award will ordinarily be presented.

Subject to Council ratification, selection of recipients shall be made by an Awards Committee consisting of five members representing the several disciplines within the Society, appointed by the President for 2-year terms. Members of the Committee shall be ineligible for consecutive appointment.

The Awards Committee may declare "no award" if unable to recommend a recipient 3 months prior to the scheduled date of presentation.

The Award shall be presented in the name of the Society at the annual general session, and it shall be deemed appropriate for the recipient to present a review of his work at that time.

PLATE I



COMPARATIVE STUDIES OF *SYPHACIA PEROMYSCI* AND *S. SAMORODINI* (NEMATODA: OXYURIDAE)*

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A study of *Syphacia peromysci* Harkema, 1936, and *S. samorodini* Erickson, 1938, was undertaken to determine characteristics which may be used to distinguish these closely related species of oxyurid nematodes. Both were described with minimal characterizations, as relatively minor portions of otherwise extensive host-parasite surveys. Additional critical studies have not been reported. Differential data are essential to continuing studies of incidence or speciation of the *Syphacia* from rodents.

S. peromysci was described (Harkema, 1936) from *Peromyscus l. leucopus* Thomas, 1895, of Durham County, North Carolina. Erickson (1938) recovered this species from the same host subspecies in Minnesota, and Tiner (1948) tentatively identified it from *P. maniculatus bairdii* Osgood, 1909, in Wisconsin. Schad (1955) discovered it in *P. maniculatus* (Bangs, 1898) from Quebec, Canada. Grundmann (1959) and Kruidenier and Peebles (1957) identified it from *P. maniculatus* of Utah and *P. m. rufinus* (Merriam, 1890) of Arizona, respectively.

S. samorodini was described from *P. l. noveboracensis* (Fisher, 1829) and *P. m. gracilis* (LeConte, 1855) of Minnesota. It has been further reported from *P. maniculatus* and *Reithrodontomys megalotis* (Baird, 1858) of Utah by Grundmann (1957).

In addition to these two species, *S. obvelata* (Rudolphi, 1902) was reported from *P. nasutus* (Allen, 1891) of Colorado by Hall (1916) and *P. maniculatus* in the state of Washington by Rankin (1945). The males of all three species possess three ventral mamelons. They are, therefore, members of the subgenus *Syphacia* of Chabaud and Biocca (1955).

Erickson (1938) did not specifically differentiate *S. samorodini* from *S. peromysci*. Presumably, he was able to make careful comparisons because he reported both species in his survey of Minnesota Cricetids and Zapodids. Tiner noted the similarity of the two species, remarking that his specimens from Wisconsin could be easily identified to either species" and "it is the writer's opinion that *S. samorodini* may be considered a synonym of *S. peromysci*" (1948, p. 89). Synonymy was apparently accepted by Khera (1954) and Hussey (1957) neither of whom included *S. samorodini* in their listings of *Syphacia*. Chabaud and Biocca (1955, p. 128) specifically placed *S. samorodini* in synonymy. Grundmann's report of *S. samorodini* implicitly rejects the synonymy (1957).

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MATERIALS AND METHODS

Specimens of *Syphacia* were taken from *P. l. leucopus* trapped in Wake County, North Carolina, by the junior author (R. Harkema). They were studied as glycerine mounts and as unstained, permanent gelatin preparations. Type specimens of *S. peromysci* are not available, but male and female type specimens of *S. samorodini* were studied (U. S. Natl. Helm. Coll. No. 9240). The latter are permanently stained whole mounts. The female is ovigerous, and the male appears sexually mature but highly contracted and coiled.

RESULTS

Data obtained from the *Syphacia* (ovigerous females and mature males) taken in Wake County and supplemental measurements of *S. samorodini* are presented in tables I and II. The data given in the original descriptions of the two species are included. The very close relationship of the syphacias, and between the two species *S. peromysci* and *S. samorodini* especially, is readily apparent. Durham County, North Carolina, the type locality for *S. peromysci* is adjacent to Wake County. Host identity, collection sites, and morphological similarities strongly indicate that the Carolina specimens studied by us are *S. peromysci.* This is assumed in the following study.

The vulva of *S. peromysci* appears to be slightly more posteriad than that of *S. samorodini* and its eggs appear to be smaller, although the largest eggs described for *S. peromysci* approximate the smallest ones produced by *S. samorodini.* The measurements obtained for *S. peromysci* were taken on 23 eggs from 10 different female specimens.

The terminal cuticular spike on the tails of *S. peromysci* males appears to be 1.4 to 2 times as long as that of the type specimen of *S. samorodini* and in no instance (30 specimens) was it hooked as in the type and as Erickson described in *S. samorodini.* The posterior mamelon of the *S. samorodini* type is larger (0.044 mm) than either the middle (0.028 mm) or anterior (0.030) mamelons as described. However, the measurements presented by Erickson (1938, p. 581) do not conform to this criterion of differentiation. The posterior mamelon of *S. peromysci* is also frequently the longest.

TABLE I. Syphacia *of* Peromyscus leucopus. *Comparative measurements in millimeters: females.*

Parasite	*S. peromysci*			*S. samorodini*
Host subspecies	*leucopus*			*noveboracensis*
Locality	Durham Co. N. C.	Wake Co. N. C.	Combined	Minnesota
No. specimens	?	10	10+	?
Total length	2.0–2.5	2.5–3.2	2.0–3.2	2.7–3.4
Maximum width	0.172	0.116–0.158	0.116–0.172	0.160–0.192
Esophagus:				
Anterior length	0.273	0.214–0.245	0.214–0.273	0.234–0.273*
Anterior width	0.046	0.037–0.042	0.037–0.046	0.045
Bulb length	0.080	0.068–0.081	0.068–0.081	0.070–0.079*
Bulb width	0.080	0.066–0.073	0.066–0.080	0.070–0.075
Total length	0.353	0.291–0.326	0.291–0.353	0.320–0.352
Excretory pore	?	0.300–0.449	0.300–0.449	0.330*
Nerve ring	0.097	0.104–0.132	0.097–0.132	0.116–0.120
Cervical alae	+	0.026–0.052	0.026–0.052	+
Cervical papillae	?	0.079–0.132	0.079–0.132	absent*
Vulva	0.507–0.700	0.530–0.634	0.507–0.700	0.424–0.475
Tail	0.525–0.700	0.264–0.418	0.264–0.700	0.576–0.648
Egg length	0.080–0.081	0.081–0.089	0.080–0.089	0.086–0.097*
Egg width	0.024	0.023–0.031	0.023–0.031	0.025–0.027

* Data taken from type specimen added to Erickson's (1938) summary.

TABLE II. Syphacia *of* Peromyscus leucopus. *Comparative measurements in millimeters: males.*

Parasite	*S. peromysci*			*S. samorodini*
Host subspecies	*leucopus*			*noveboracensis*
Locality	Durham Co. N. C.	Wake Co. N. C.	Combined	Minnesota
No. specimens	?	10	10 +	?
Total length	0.913–1.3	0.64–1.10	0.64–1.3	0.5–0.74*
Maximum width	0.120–0.214	0.068–0.102	0.068–0.214	0.072
Esophagus:				
Anterior length	0.140–0.150	0.092–0.158	0.092–0.158	0.072–0.133*
Anterior width	0.030	0.021–0.029	0.021–0.030	0.028
Bulb length	0.066–0.080	0.040–0.058	0.040–0.080	0.026–0.042*
Bulb width	0.066–0.080	0.039–0.052	0.039–0.080	0.026–0.042*
Total length	0.206–0.230	0.132–0.216	0.132–0.230	0.098–0.175*
Excretory pore	0.172	0.132–0.274	0.132–0.274	0.096*
Nerve ring	0.090	0.064–0.071	0.064–0.090	0.42
Cervical alae	+ (0.060?)	0.023–0.052	0.023–?	+
Cervical papillae	0.060(?)	absent	absent	absent
Tail (less spike)	?	0.023–0.031	0.023–0.031	0.027*
Cuticular spike	?	0.018–0.026	0.018–0.026	0.013*
Spicule length	0.070	0.058–0.070	0.058–0.070	0.058–0.068
Spicule width	0.003–0.004		0.003–0.004	0.002
Gubernaculum length	0.037	0.026–0.031	0.026–0.037	0.026–0.031*
Gubernaculum width	0.005			0.004*
Accessory piece	0.020	0.015–0.018	0.015–0.020	0.018*
Mamelon anterior	0.050	0.039–0.060	0.039–0.060	0.031–0.042
Mamelon medium	0.050	0.039–0.052	0.039–0.052	0.031–0.042
Mamelon posterior	0.050	0.044–0.058	0.044–0.058	0.029–0.056
Distance between mamelons		0.316–0.461	0.316–0.461	
Circumanal papillae	?	4	4	(4?)

* Data taken from type specimen added to Erickson's (1938) summary.

Differences in basic structure are also present. The vagina (fig. 2) of *S. samorodini* appears conspicuously the more muscular. It is directed across the entire body of the female and at approximate right angles to the linear axis. In the more than 50 *S. peromysci* females studied in various stages of contraction and coiling, the vagina of *S. peromysci* (fig. 1) always curves anteriad in the median to ventral portion of the body before turning to enter the vulva and it is always relatively inconspicuous (i.e., not as highly muscularized).

All of the females in the Wake County collection of *S. peromysci* possess a pair of bilaterally symmetrical, cervical papillae. The latter attain about 0.011 mm length and are located beyond the termination of the narrow cervical alae. A group of fiber tracts (about four) extend between the nerve ring and the papillae. They originate at the nerve ring (fig. 3). The papillae are, therefore, sensory. They are rather difficult to see in laterally oriented specimens. None were visible in males of *S. peromysci* and they can not be seen in either of the type specimens of *S. samorodini.*

Most of the male *S. peromysci* studied were longer than those of *S. samorodini* reported by Erickson, but the few smaller specimens obtained indicate that size is not a good criterion. Similarly, while the esophagus in males of *S. peromysci* seemed generally longer, a few specimens compared closely with the range in measurement noted for *S. samorodini.* The single measurement for the excretory pore or *samorodini* (type specimen) is inaccurate because of the considerable contraction of the anterior end of the individual.

The males of each species possess an accessory piece fused to the distal end of the gubernaculum although the former structure has not been reported previously in *S. samorodini.* The size and general configuration (figs. 4 and 5) of this structure appears identical in both species. In each it is pointed sharply and recurved

into the shape of an attenuate S. The greatest curvature is distal, at the unattached end near the cloaca. Strong strands of retractile muscles attach to the proximal portion. The ventrolateral surfaces of each accessory piece have a series of minute, bilaterally paired, blunted projections. Seven pairs of the projections were found in the accessory piece of *S. samorodini* (type specimen). The accessory piece of *S. peromysci* possesses five pairs of the projections in most specimens (25) but appears variable (three pairs in one specimen). The most anterior and posterior projections in each case are smaller than the others. They are difficult to discern in poorly oriented specimens and could not be counted in several instances.

Apparently similar minute projections were described in *S. thompsoni* Price, 1928, as post-cloacal, surface structures. Li (1933; p. 1322) placed these on the "distal end of the gubernaculum." The drawings (Price, 1928, pl. 1, fig. 3, Li, 1933, pl. 1, fig. 9a) indicate that the structures observed in *S. thompsoni* are identical to the projections ascribed to the accessory piece in *S. peromysci* and *S. samorodini*.

ACKNOWLEDGMENT

Gratitude is expressed to Miss Alice Boatright, Scientific Illustrator, Department of Zoology, University of Illinois, for aid in the preparation of the camera lucida drawings.

SUMMARY

The close similarity of *S. samorodini* to *S. peromysci*, which has led to designations of synonymy for the latter, is very apparent. Their small size doubtless emphasizes the similarities expected of closely related species. However, it appears possible to distinguish the females of the two species by means of the cervical papillae, the relative position of the vulva, the strength and position of the vagina, and the range in size of the eggs produced. The detailed morphology of the accessory piece, the length of the esophagus, the length, and possibly the shape of the cuticular spike of the tail appear to differentiate the males of the species. It would appear best at present to retain both species. Further studies of *S. samorodini* from Minnesota should be undertaken to determine the constancy of the observed differential factors in that species.

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EXPLANATION OF PLATE I

FIGURE 1. *Syphacia peromysci:* Vagina
FIGURE 2. *S. samorodini:* Vagina
FIGURE 3. *S. peromysci:* Female, cervical papillae
FIGURE 4. *S. peromysci:* Male, posterior and lateral view
FIGURE 5. *S. samorodini:* Male, posterior and lateral view

RESEARCH NOTE

PARASITES OF IDAHO REPTILES.

During the summers of 1957 and 1958, fifty-seven reptiles representing 15 different species were collected and examined for parasites. Two species of trematodes, six species of cestodes, and four species of nematodes were recovered.

The hosts examined and numbers of each follow: 5 *Chrysemys picta*, 1 *Crotaphytus collaris*, 5 *Crotaphytus wislizenii*, 9 *Sceloporus graciosus*, 5 *Sceloporus occidentalis*, 2 *Uta stansburiana*, 3 *Phrynosoma platyrhinos*, 2 *Gerrhonotus coeruleus*, 1 *Cnemidophorus tigris*, 7 *Eumeces skiltonianus*, 7 *Thamnophis elegans*, 7 *Thamnophis sirtalis*, 1 *Coluber constrictor*, 1 *Masticophis taeniatus*, and 1 *Pituophis catenifer*.

The parasites found and their hosts follow.

TREMATODA

Lechriorchis plesientera Sumwalt, 1926, in the esophagus of one *Thamnophis elegans* and one *Thamnophis sirtalis*.

Telorchis bonnerensis Waitz (in press), in the intestine of one *Thamnophis sirtalis*.

CESTODA

Oochoristica natricis Harwood, 1932, in the intestines of two *Crotaphytus wislizenii*.

Oochoristica scelopori Voge and Fox, 1950, in the intestines of two *Sceloporus graciosus*.

Oochoristica fibrata Meggitt, 1927, in the intestine of one *Coluber constrictor*.

Oochoristica sp. in the intestine of 5 *Eumeces skiltonianus*.

Ophiotaenia perspicua in the intestines of two *Thamnophis elegans* and one *Thamnophis sirtalis*.

NEMATODA

Cyrtosomum readi Gambino, 1958, in the large intestines of three *Crotaphytus wislizenii* and one *Phrynosoma platyrhinos*.

Skrjabinoptera phrynosoma (Ortlepp, 1922) in the large intestines of one *Phrynosoma platyrhinos* and one *Sceloporus occidentalis*.

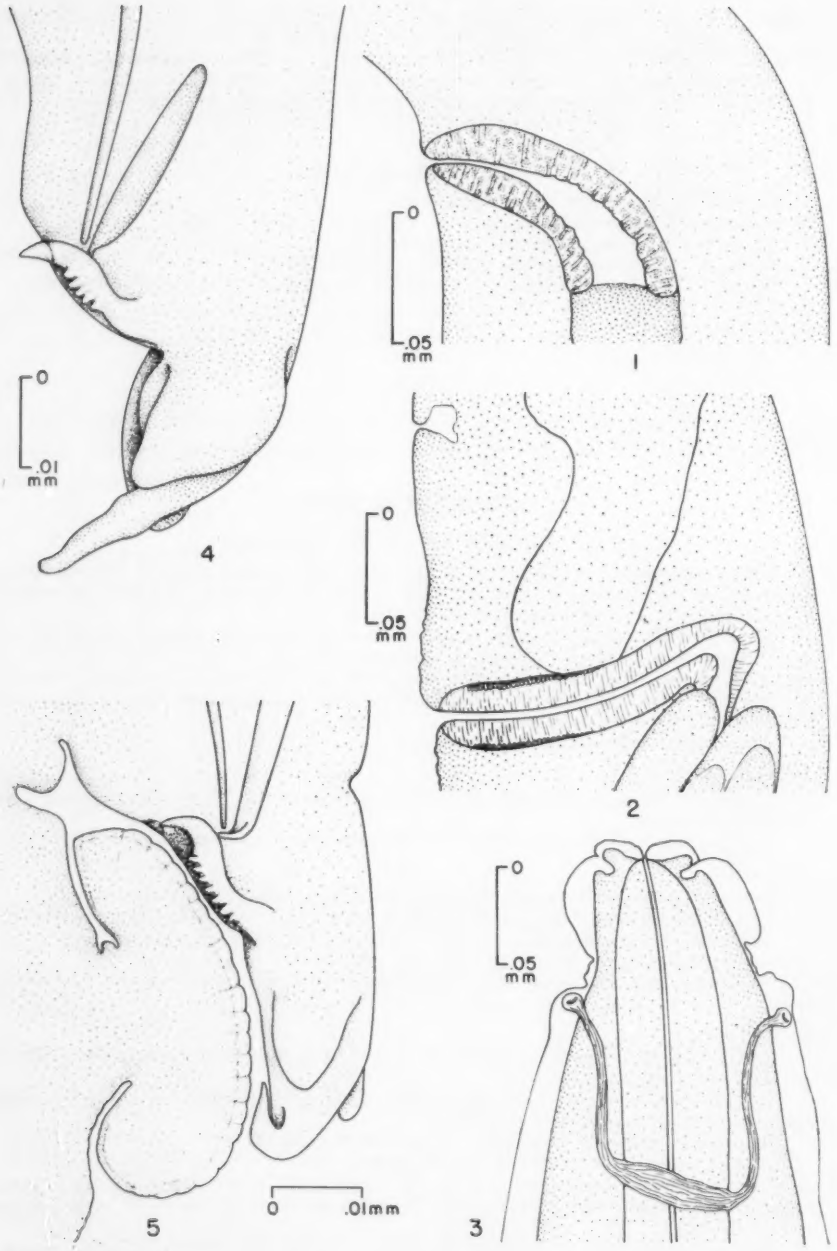
Physaloptera abjecta in the intestine of one *Coluber constrictor*.

Oxyurid larvae in the intestine of one *Thamnophis sirtalis*.

Chrysemys picta, Crotaphytus collaris, Uta stansburiana, Gerrhonotus coeruleus, Cnemidophorus tigris, Masticophis taeniatus, and *Pituophis catenifer* were not found to be infected with helminths.

This investigation was supported in part by a grant from the Northwest Scientific Association and was completed under the tenure of a fellowship (EF-9587) from the U. S. Public Health Service.—J. ALLAN WAITZ, *Department of Zoology, University of Illinois, Urbana, Illinois.*

PLATE I



HYMENOLEPIS DIMINUTA IN SURGICALLY ALTERED HOSTS. I. ATTEMPTS TO CULTIVATE CESTODES IN ISOLATED SECTIONS OF THE SMALL INTESTINE: OBSERVATIONS ON CHANGES IN THE ISOLATED AND REJOINED INTESTINES*

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In vitro cultivation of cestodes to sexual maturity, except for precocious plerocercoids of certain pseudophyllideans (Smyth, 1950, 1954), has failed because of dearth of information regarding their basic nutritional and environmental requirements. It seems desirable, therefore, to continue extensive studies on the intestine and the parasite in vivo. Information gained from such studies may lead to a more rational approach to their in vitro maintenance.

Previous studies on in vivo physiology of host and parasite have been made, in part, by: (1) administering drugs to affect peristalsis or metabolism (Larsh, 1947, 1950; Read and Voge, 1954); (2) modifying the host's diet (Shorb, 1933; Chandler, 1943; Addis and Chandler, 1944, 1946; Chandler, Read and Nicholas, 1950; Beck, 1951; Read and Rothman, 1957a, b, c); (3) altering hormonal secretions (Addis, 1946; Beck, 1952); and (4) surgical modification of the digestive tract (Goodchild, 1954, 1957, 1958a, b; Goodchild and Boodish, 1955).

In the surgical approach to this problem a section of the small intestine was isolated from the rest of the tract, both sections having their vascularization intact, the so-called Thiry-Vella fistula. The isolated loop should theoretically result in a habitat free of exogenous food but provided, perhaps, with nutrients supplied by the vessels associated with the isolated section.

In the present study, different regions of the small intestine were isolated; in some isolates the flow of bile was prevented while into others bile was permitted to flow normally via the bile duct or caused to discharge into them by a cannula.

The study was designed to determine the effects of the isolated section of the intestine upon the parasite, to determine the effects of the surgical technique upon the isolated section itself and upon the reconstituted intestinal tract, and to determine the gross effect of the operation upon the host. Adult *Hymenolepis diminuta,* obtained from donor Wistar rats, were implanted into the isolates to determine their survival and possible morphological and biochemical alterations. In addition, changes in the intestinal isolates, in length and diameter, and noticeable expressions of physiological alterations in the isolated and rejoined intestinal tract were ascertained. Finally, the effect upon weight changes and gross appearance of the altered host were also studied.

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MATERIALS AND METHODS

Eighty-four Wistar rats, between 200 and 350 g at time of operation, were used. Rats were housed individually in wire cages provided with automatic water dispensers and fed ad libitum with Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.). The temperature of the animal house was maintained at about 25 C.

The strain of *H. diminuta* was originally obtained from the Rice Institute and has been maintained in serial culture for several years at Emory University. Cysticercoids were obtained using the technique of Goodchild (1958a) and were fed to rats within 3 hours after sacrificing the host beetles (*Tribolium confusum*). Adult tapeworms used for implantations were obtained by perfusing, with warm Locke's solution, the small intestine of donor rats infected 14 days previously.

All surgery was effected using clean but not aseptic techniques. Preparatory to operation the animal was lightly etherized and weighed. A portion of its right flank, from just behind the rib cage to the inguinal area, was shaved and the area cleansed with 70 percent ethanol. While keeping the animal under light ether anesthesia, a primary incision was made through the dermal and subdermal layers, extending from just posterior to the ribs to within about 2 to 3 cm of the hind leg. After packing the area with sterile gauze pads soaked with sterile mammalian Locke's solution, the intestine was delivered out of the body cavity. The intestinal diameter was measured with a sterile ruler and the length with the aid of a sterile string. After measurements were taken, selected regions of the intestines were returned to the body cavity and the portions to be transected were left on the packing gauze which was folded over them to prevent desiccation. The intestine was exposed only for measuring, operating, or taking photographs.

Intestinal areas were isolated at various levels (figure 1). The vascularization of isolates was left intact except for minor tributaries which were electrocauterized at the levels of transections; the remaining intestine was rejoined to form a functional gut. Anterior transections of type A were made anterior (figs. 1a, 1b, 1c) or posterior (figs. 2a, 2b) to the entrance of the bile duct; posterior transections of type A were made at levels to include in the isolates the anterior one-third, one-half, or two-thirds of the total small intestine. The posterior ends of type A isolates were joined to the apex of the caecum. A tapeworm was then implanted; the anterior end of the isolate was blinded with a Parker-Kerr closure and attached to the body wall. Twenty-six rats were used in the A series and were sacrificed and examined after 16 hours to 28 days following implantation. Type B isolates utilized the middle third of the intestine and were made with two variations: (1) The isolate was flushed with warm saline, a tapeworm was implanted, and the section returned with both ends open to the body cavity; and (2) the posterior end of the isolate was rejoined to the caecum, a tapeworm was implanted and the anterior end was closed and attached to the body wall. Twenty-four rats, in two equal groups, were operated in the B series and were sacrificed and examined after 1 to 22 days following implantation. Type C isolates of the posterior half of the small intestine were made with two variations: (1) The anterior end was blinded and attached to the body wall; and (2) the anterior end of the isolate was closed and attached to the body wall, as before, but a cannula rerouting bile to the isolated half was surgically implanted. Implantation of tapeworms in type C isolates was either at time of the operation or 7 days later by a second operation. Thirty-four rats were used in the C series of which 18 were cannulated. Sacrifice and examination occurred from 6 hours to 12 days following implantation.

For rejoining the intestinal sections to form a functional intestinal tract serosa-to-serosa apposition was used following the method of Carrell (1907). For joining intestinal sections to the caecum, an opening was made in the apex of the caecum which was then reduced by a drawstring suture; the intestine was then stitched into the reduced opening. All tapeworms were implanted into the isolates, immediately after removal from donors, by means of a short length of narrow polyethylene tubing attached to a 5-ml hypodermic syringe.

To cannulate the bile duct, the pancreas and stomach were displaced and the duct ligated at the point of entry into the small intestine and allowed to distend by the accumulation of bile. A length of PE 50 polyethylene tubing was then introduced via a nick made in the duct at a point close to the ligature; the tubing was carefully introduced to the bifurcation of the duct close to the liver. The cannula was tied snugly in place, the free end was cut to the appropriate length, and, after ascertaining that bile was flowing freely, sutured into the lumen of the isolate.

After all transections, cannulations, implantations, and intestinal suturing were completed, the viscera were returned to the body cavity and the primary incision repaired with 5-0 silk thread. The area was cleaned with warm distilled water, dried with gauze, and anointed with 5 percent sulfathiazole. In addition, 0.5 ml of S-R-D antibiotic (Parke, Davis and Co., Detroit, Mich.) was injected intramuscularly in the general area of the incision. Rats were then returned

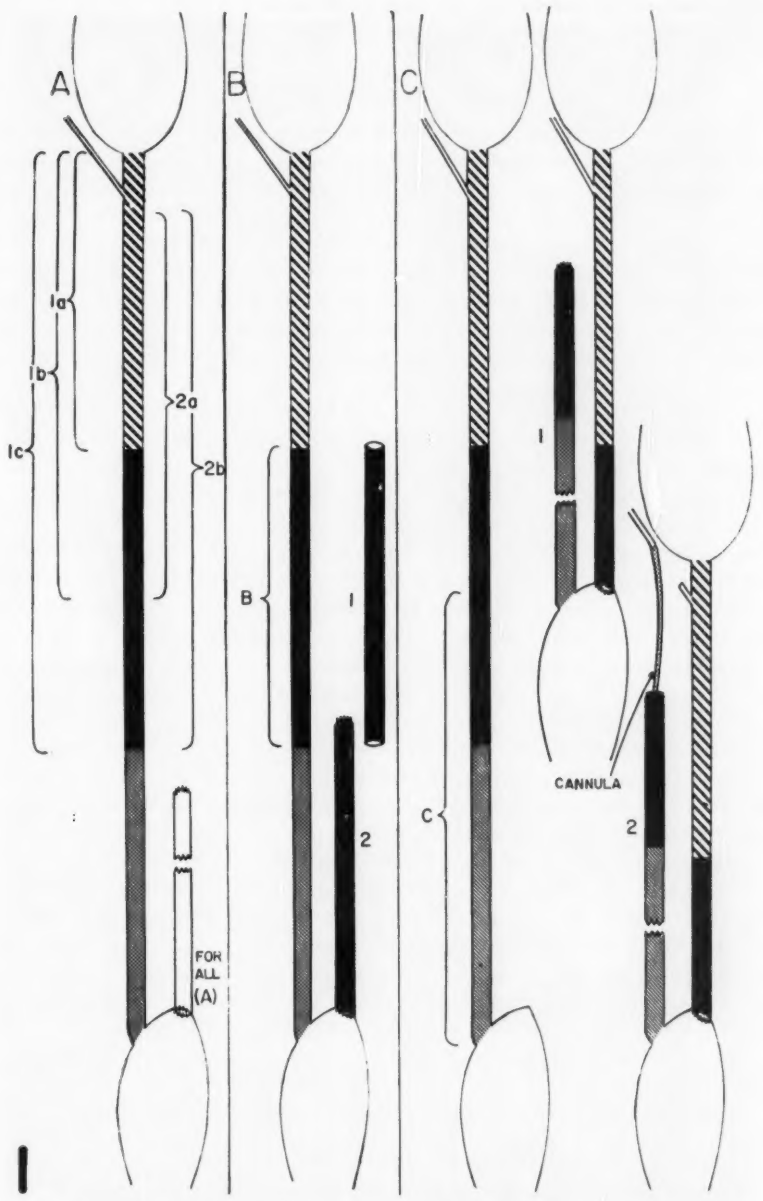

FIGURE 1. Diagrams of operations used in the experiments. Stomach represented at top, small intestine (with entrance of bile duct at top left) represented by middle section, large intestine (with caecum) represented at bottom. Type A illustrates anterior isolates with and without bile; Type B represents middle third isolates; Type C illustrates posterior-half isolates with (cannulated) and without bile.

to their cages and kept without food and water for 24 hours.

At various times after the operations and implantations rats were sacrificed and data recorded.

OBSERVATIONS AND RESULTS

Living tapeworms were not recovered from any of the isolated portions of the small intestine. Rats were sacrificed from 6 hours to 30 days post-implantation, but even the earliest recovered implants were dead and embedded in mucus which occluded the lumen in all isolates deprived of bile (type A: 2a, 2b; type B; and type C: 1). Beyond 6 hours dead worms were more solidly entrapped in mucus and were undergoing histolysis. After about 2 to 3 days the implants had completely disintegrated and even though the packed mucus was thoroughly teased no trace of tapeworm was found. Figure 2 illustrates the occlusion of the lumen and a trapped worm (7 days post-implantation), and figure 3 shows the compactness of the same mucus strand after removal from the isolate.

Lumina of isolated sections provided with bile (type A: 1a, 1b, 1c; and type C: 2) were devoid of solidified mucus, but like those above, failed to support survival of implanted rat tapeworms.

The isolated section of intestine exhibited rhythmical peristalsis and appeared to have an adequate blood supply to keep the tissues in good condition. Furthermore, the lumina of isolates were devoid of traces of small quantities of exogenous food present in the gut at the time of operation. Whether this indicates digestion by enzymes in the succus entericus or by bacteria has not yet been determined. Moreover, pH determinations were not made of the luminal contents of the isolates.

In all three major types of isolated sections, and in the reconstituted intestine, a rather remarkable and consistent morphological pattern was observed. The lengths and diameters of isolated and rejoined intestines differed at time of sacrifice from measurements taken at the time of operation. It was noted that in all isolates lengths and diameters had decreased; whereas in the reconstituted intestines lengths had increased in all three types, but increased diameters were correlated to presence or absence of bile. The increase in diameter was most pronounced in the reconstituted intestine deprived of bile. Figure 4 illustrates the distal half of a small intestine (type A: 2a) made by reconstituting at the duodenal stump just posterior to the entry of the bile duct and hence having a normal flow of bile. Measurements of this rejoined gut were 44 by 0.6 cm (length and diameter) at time of the operation, and 55 by 0.7 cm after 5 days. On the other hand, reconstituted intestines deprived of bile, had increases in length similar to those given above, but diameters increased nearly three-fold over initial measurements, (fig. 5). Measurements of the bileless rejoined intestine were 38 by 0.6 cm initially and 50 by 1.5 cm after 5 days.

In addition to changes in length and diameter the reconstituted intestine showed

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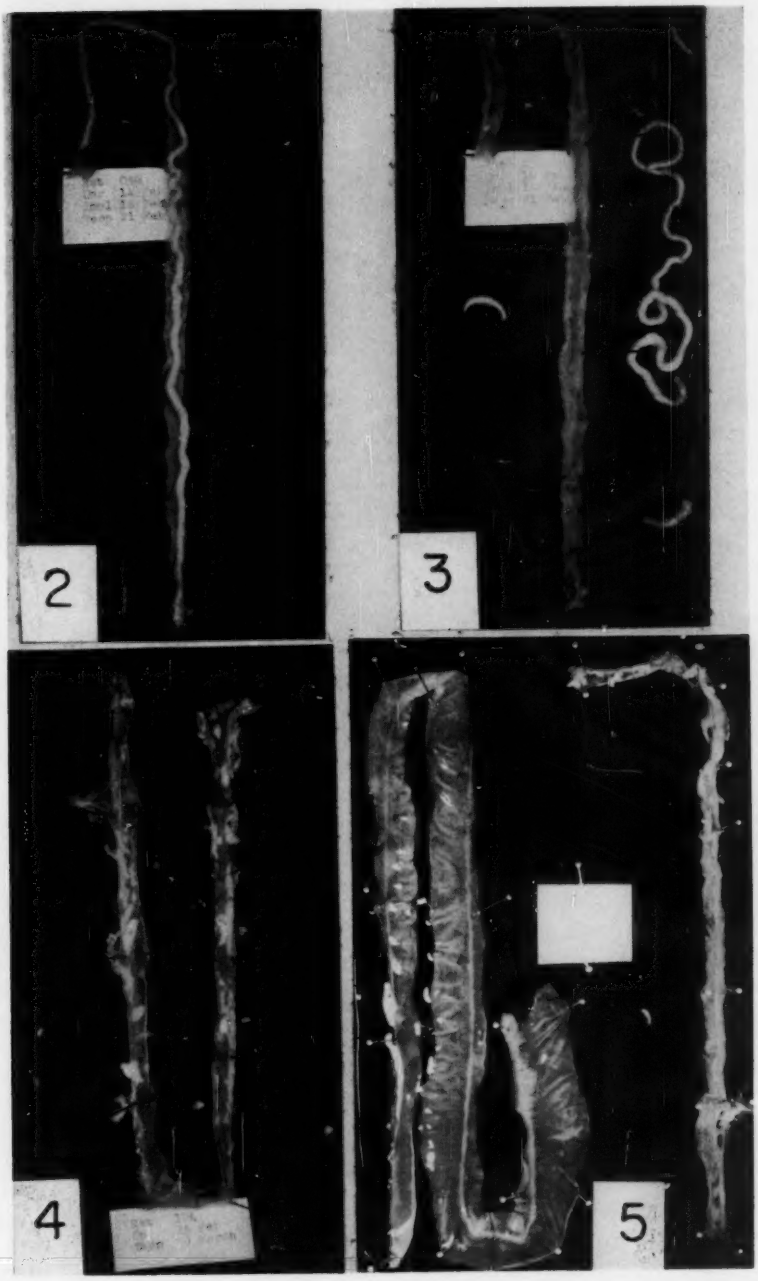
FIGURE 2. Occluding mucus mass in isolated section of small intestine, in situ.

FIGURE 3. Occluding mucus mass removed from isolated section of small intestine.

FIGURE 4. Reconstructed section of small intestine into which bile flowed. Section had increased slightly in length and diameter.

FIGURE 5. Figure on left: reconstructed section of small intestine lacking bile. Note large increase in length and diameter as compared to figure 4. Figure on right: Isolated intestinal section which showed decrease in length and diameter as compared to original measurements.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morphological alteration in the gross appearance of the wall. The rejoined gut with normal bile flow appeared similar to the normal intestine; whereas, the reconstituted intestine without bile was thin-walled and flabby. In all types of operations, moreover, the rats continued to eat to maintain weight, and appeared sleek and adequately nourished.

DISCUSSION

Hymenolepis diminuta failed to survive in isolated sections of the small intestine. Death was prompt; in fact, it occurred more rapidly within the isolates than in Kreb's-Ringer phosphate or bicarbonate solutions, where survivals may exceed 50 hours (unpublished data), or in more complex artificial media in roller tubes, in which survival for 11 days has been reported (Schiller et al, 1959). The cause for this rapid death in vivo was not determined. In isolates devoid of bile, worms became quickly embedded in mucus and were displaced into the lumen and away from their normal mucosal apposition. However, in cannulated isolates, devoid of mucus, worms were nevertheless dead and undergoing histolysis approximately 6 hours after implantation. The nature of the cannulation in type C: 2 isolates unfortunately permitted pancreatic secretions to mix with the bile. In fact, the proteolytic activity of this pancreatic juice may be responsible for the differences between results obtained in this experiment, where death of implanted tapeworms ensued within 6 hours, from those reported earlier (Goodchild, 1954), where survival of tapeworms for 3 days was obtained in the Thiry-Vella fistula.

Survival of *H. diminuta* for 3 days in Thiry-Vella fistulas of Wistar rats (Goodchild, 1954) seemed to provide a technique for investigating the enteric habitat of adult tapeworms. To date, however, over 100 rats have been fistulated and have been implanted with adult *H. diminuta* in different types of experiments (unpublished), but survival of these implants beyond 3 days has not been achieved. The results in these experiments have been approximately the same as reported above. Worms behaved normally for a day or two and would actively crawl back into the fistular opening when flushed with warm saline to remove the entrapping mucus, but by day 3 they were dead or were absent when the rat was sacrificed and the fistula opened. Although the Thiry-Vella fistula was an incompatible habitat for *H. diminuta* it was suitable for *Trichinella spiralis* since juveniles implanted into the fistula became sexually mature and established viable encysted juveniles in the skeletal muscles of the rat (Goodchild, 1957). A plausible explanation for these differences in behavior between *H. diminuta* and *T. spiralis* may be the saprozoic nutrition of the tapeworm, which may be more sensitive to changes in the environment, as compared to the predaceous nature of *T. spiralis* which subsists upon tissue fluids ingested from cells of the intestinal wall.

Goodchild (1958b) reported that *H. diminuta* usually recovered promptly when removed from the donor host and transplanted into the normal intestine of recipient rats. In some cases, however, especially when implantation was made in the lower ileum, worms were carried into the colon before they could make contact with the intestinal wall and perished. In the present study, when implantation was made at the time of operation, an additional complicating factor may have been introduced, that of increased peristalsis. According to Portis (1953), conditions causing intestinal irritation result in increased peristalsis. Thus, worms implanted during the operation may have been swept into the colon before their recovery from the im-

plantation. Nevertheless, this is not the complete explanation for lack of survival, since worms implanted 7 days after the primary operation by a very minor surgical manipulation, when presumably peristalsis should have returned to normal levels, were all dead within 24 hours. Failure of survival of worms in the present study seemed not to be due to errors in experimental techniques. All worms except two were implanted immediately after their removal from donors; the two exceptional ones were implanted within 3 hours. Worms appeared to be alive and normal at the time of implantation. Furthermore, identical techniques (Goodchild, 1958a) yielded normal worms from normal recipient hosts in transfaunations performed simultaneously with these experimental ones.

Changes in length and diameter of isolated and rejoined intestinal sections appear, in the latter at least, to be an effort on the part of the host to compensate for decreased area in the rejoined intestine. Histological examination of the rejoined intestines has not yet been made; it is not known, therefore, whether the increases were accomplished by movements of cells in the original wall or by increases in numbers of cells. Striking increases in diameter in bileless rejoined intestines, as compared to those with a normal bile flow, are as yet unexplained, but should provide an excellent model system for investigation of this striking compensation.

SUMMARY

A study was undertaken to determine whether adult rat tapeworms, *Hymenolepis diminuta,* could be successfully transplanted and maintained in isolated sections of the small intestine of Wistar rats. The isolates cut from various regions were provided with a normal blood supply, but were devoid of exogenous food; the remaining small intestine was surgically rejoined to form a functional gut.

Results indicated that adult *H. diminuta* do not survive transplantation into various modifications of intestinal isolates. In isolates devoid of bile, worms became quickly embedded in secreted mucus that formed rapidly and occluded the lumen; in isolates provided with bile the mucus mass did not form, but worms promptly died and underwent histolysis.

Isolated sections of the intestine decreased in length and diameter, whereas the reconstituted small intestine elongated and increased in diameter. In rejoined intestines where bile was absent the increase in diameter was more pronounced than it was in those where bile was present.

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RESEARCH NOTE

THE OCCURRENCE OF *RHOPALIAS MACRACANTHUS* (TREMATODA: RHOPALIASIDAE) IN THE WILD TURKEY (*MELEAGRIS GALLOPAVO*).

Four species of flukes of the genus *Rhopalias* have been described. Three species are indigenous to South and Central America and one is native to North America; all are intestinal parasites of various opossums, *Didelphis* spp. (Yamaguti, S., 1958, Systema Helminthum **1**: 901).

The North American species, *R. macracanthus*, was described from *Didelphis virginiana* from Houston, Texas (Chandler, A. C., 1932, Proc. U. S. Natl. Mus. **81** (16): 1–15). According to S. Yamaguti (1958, Systema Helminthum **1**: 901) it has since been reported from *D. mesamexicana tabascensis* from Mexico. This paper reports *R. macracanthus* from a wild turkey.

A single adult fluke containing mature eggs was recovered from the intestine of a wild turkey taken 5 miles southwest of Pretoria, Georgia, on January 22, 1957. The fluke was smaller than specimens examined by Chandler from *D. virginiana*, but otherwise identical to his description of the species. The specimen from the turkey measured: total length, 320 mm; diameter of anterior body, 0.59 mm; length of proboscis sac, 0.25 mm; length of proboscis spines, 0.107 mm. The ceca were visible throughout their length and extended to within 0.187 mm of the posterior end of the body. Chandler was unable to follow the ceca of his specimens beyond where they reached the vitellaria.

Although the fluke had developed to sexual maturity in the turkey, its small size probably indicates that it was not living in its optimum environment.—STURGIS MCKEEVER, *Department of Zoology, University of California, Davis.*

THE USE OF FORMALINIZED RED CELLS IN THE SEROLOGY OF HYDATID DISEASE

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Boyden's hemagglutination technique (Boyden, 1951) employing antigen adsorbed onto tanned fresh red cells has been used routinely in our laboratory in the serologic diagnosis of various parasitic infections (Kagan and Oliver-Gonzalez, 1958; Kagan et al, 1959a, 1959b). Certain modifications of this method have proved advantageous, particularly the use of red cells which have been stabilized by treatment with formalin. This report is an evaluation of the stability of formalinized sheep red cells which have been sensitized with echinococcus antigen and stored at 5 C and – 70 C in antigen suspension. Additional lots of these antigen-sensitized cells were also dried by lyophilization, stored at – 20 C and reconstituted prior to use.

MATERIALS AND METHODS

Formalinized sheep red blood cells were prepared according to a modified version of Csizmas' method (Csizmas, 1960). Fresh sheep cells were collected aseptically by bleeding into a flask containing one volume of modified Alsever's solution (Bukantz et al, 1946) per volume of blood. The cells were then washed five times with ten volumes of cold physiological saline and centrifuged in the cold at 1000 *g* for 15 minutes. After the last washing, the packed cells were resuspended in eight volumes of cold saline. Formalin (40 percent USP formaldehyde) was adjusted to a pH of 5.5 to 6.0 with 1.0 N NaOH, and two volumes of the formalin based on the original volume of packed cells were poured into dialysis tubing, allowing a 50 percent air space for expansion, and placed in a beaker. The freshly washed cells were poured over the dialysis tubing containing the formalin, and the beaker was rotated on a rotary shaker in the refrigerator for 1.5 hours. Care was taken to prevent foaming by adjusting the speed of the shaker. After 1.5 hours the dialysis tubing was cut, the formalin allowed to run directly into the beaker of cells, and the suspension shaken overnight in the refrigerator. The formalin was then removed by washing the cells 10 times with cold saline. The packed cells were diluted to a 25 percent suspension in merthiolated saline (1 : 10,000) and stored in the refrigerator until use.

Echinococcus antigen, prepared from hog hydatid fluid, was used for sensitizing the cells (Kagan et al, 1959a). A sample of the stock formalinized cells was washed four times with buffered saline (pH 7.2) and adjusted to a 2.5 percent suspension. An equal volume of 1 : 20,000 tannic acid in buffered saline was added and the mixture incubated in a water bath at 37 C for 1 hour. The tanned cells were then washed twice with buffered saline, and sensitized by adding five volumes of antigen per volume of packed cells. The antigen-cell suspension was allowed to stand overnight in the refrigerator before testing and further processing.

Immediately before testing, a sample of the sensitized cells was washed 3 to 6 times with buffered saline to remove all excess antigen. Sera of known titer were diluted in two-fold serial dilutions beginning with 1 : 50 and tested by the hemagglutination technique reported previously (Kagan et al, 1959a).

To determine the stability of the antigen-cell suspension, separate lots were stored at 5 C and – 70 C. Identical suspensions of formalinized, sensitized cells in an excess of antigen were freeze-dried for 6 hours and stored at – 20 C. In the first experiment, seven lots of antigen-cell suspensions stored at 5 C were tested with three human sera (one negative and two positive for echinococcosis) at intervals up to 3 weeks or until found unsatisfactory. Samples of each lot were washed six times before testing.

In the second experiment, one small lot of formalinized, sensitized cells was frozen and stored at – 70 C and tested at intervals up to 6 months.

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In the third experiment, two lots of formalinized, antigen-coated cells were processed by freeze-drying for 6 hours and stored at −20 C. The first lot was tested at 1-week intervals for a period of 3 weeks. The second lot was tested once each month for 7 months and is still being evaluated.

RESULTS

The results of hemagglutination tests using sensitized cells after storage at 5 C are shown in table I. Titers obtained with fresh cells for serum E3 was 1 : 6400 ± two dilutions and for serum E4, 1 : 204800 ± two dilutions. One batch of cells was satisfactory for 1 week, five lots of cells were satisfactory for 2 weeks, and one for 3 weeks. The low titers obtained with lot 6 are attributed to the use of a weak antigen in the preparation of the cells. In general, it may be concluded, that formalinized sensitized cells stored in the refrigerator may be used for approximately 2 weeks.

TABLE I. *Hemagglutination titers of seven lots of formalinized sheep red cells stored at 5 C.*

	Sera Tested		
	Negative	E-3	E-4
LOT 1			
1st week	0	1 : 6400	1 : 204800
2d week	0	1 : 3200	1 : 102400
3d week		1 : 12800	1 : 409600 +
LOT 2			
1st week	0	1 : 6400	1 : 204800
2d week	0	1 : 25600 +	1 : 409600 +
3d week	AGG*	AGG	AGG
LOT 3			
1st week	0	1 : 6400	1 : 102400
2d week	0	1 : 6400	1 : 25600
3d week	AGG	AGG	AGG
LOT 4			
1st week	0	1 : 800	1 : 51200
2d week	0	1 : 6400	1 : 204800
3d week	1 : 400	1 : 6400	1 : 51200
LOT 5			
1st week	0	1 : 6400	1 : 51200
2d week	AGG	AGG	AGG
3d week	AGG	AGG	AGG
LOT 6			
1st week	0	1 : 800	1 : 25600
2d week	0	1 : 800	1 : 51200
3d week	AGG	AGG	AGG
LOT 7			
1st week	0	1 : 6400	1 : 204800
2d week	0	1 : 12800	1 : 51200
3d week	AGG	AGG	AGG
FRESH CELLS	0	1 : 6400 ± 2 titers	1 : 204800 ± 2 titers

* AGG, agglutinated.

The results of the second experiment (table II) indicate that formalinized, sensitized antigen-coated cells frozen and stored at −70 C were satisfactory after 6 months of storage.

TABLE II. *Hemagglutination titers of formalinized-sensitized sheep red cells stored at −70 C.*

	Sera Tested		
	Negative	E-3	E-4
LOT 1			
1st month	0	1 : 12800	1 : 409600
3d month	0	1 : 12800	1 : 204800
6th month	0	1 : 25600	1 : 204800
FRESH CELLS	0	1 : 6400 ± 2 titers	1 : 204800 ± 2 titers

The results of the third experiment wherein the formalinized sensitized antigen-coated cells had been freeze-dried and stored at – 20 C are shown in table III. It is

TABLE III. *Hemagglutination titers of 2 lots of formalinized sheep red cells freeze-dried and stored at – 20 C.*

	Sera Tested		
	Negative	E-3	E-4
LOT 1*			
1st week	0	1 : 6400	1 : 102400
2d week	0	1 : 12800	1 : 102400
3d week	0	1 : 12800	1 : 102400
LOT 2**			
1st month	0	1 : 3200	1 : 204800
2d month	0	1 : 6400	1 : 204800
3d month	1 : 400	1 : 6400	1 : 409600
4th month	0	1 : 6400	1 : 102400
5th month	0	1 : 6400	1 : 204800
6th month	0	1 : 6400	1 : 409600
7th month	0	1 : 3200	1 : 102400
FRESH CELLS	0	1 : 6400 ± 2 titers	1 : 204800 ± 2 titers

* Titers of sera with cells of Lot 1 before freeze-drying: Neg—0, E-3 1 : 6400, E-4 1 : 204800.
** Titers of sera with cells of Lot 2 before freeze-drying: Neg—0, E-3 1 : 1600, E-4 1 : 102400.

evident that after 7 months of storage, the samples reconstituted and tested gave results consistent with those obtained using fresh cell suspensions.

DISCUSSION

Several methods for formalinization of red blood cells for use in hemagglutination tests have been published (Flick, 1948; Cox, et al, 1956; McKenna, 1957, Ingraham, 1958, Weinbach, 1959). The relative ease with which sheep cells can be prepared by Csizmas' technique makes it the method of choice in our laboratory. McKenna (1957) and Weinbach (1959), have previously reported that formalinized non-sensitized and formalinized cells sensitized with bacterial antigens could be freeze-dried without apparent alteration, and that reactions obtained with these cells were the same as those with fresh cell suspensions. Dr. Csizmas had also told us that formalinized red cells would withstand lyophilization which led to our evaluation of this method.

The use of formalinized cells sensitized with echinococcus antigen has several advantages over fresh cells in the hemagglutination technique. The cells remain stable for relatively long periods of time so that they may be used readily in a routine diagnostic laboratory. The use of dried cells suspensions which have been sensitized and coated with antigen makes possible the preparation of reference standard antigens for use by other laboratories. Other parasitic antigens are now being tested with formalinized cells in the hemagglutination technique, and promising results have been obtained with schistosome antigens.

SUMMARY

Formalinized sheep red cells, sensitized with tannic acid and coated with echinococcus antigen, can be substituted for fresh cells in the hemagglutination test. Antigen coated formalinized cells stored at 5 C were stable for 2 weeks, and for 6 months when stored at – 70 C. Antigen coated formalinized sheep red cells freeze-dried and stored at – 20 C were satisfactory after being reconstituted for use in the hemagglutination test after 7 months of storage.

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RESEARCH NOTE

ANOTHER POTENTIAL INTERMEDIATE HOST SNAIL FOR *SCHISTOSOMA MANSONI* IN PUERTO RICO.

Attempts to infect Puerto Rican snails other than *Australorbis glabratus* with *Schistosoma mansoni* generally have failed to result in development of the parasites to the stage of cercarial production. Laboratory infection of snails considered to be *Tropicorbis riisei* with subsequent release of cercariae of *S. mansoni* is here reported.

A collection of planorbid snails identified morphologically as *T. riisei* (which may be the same as *Tropicorbis peregrinus* and *T. havanensis*) was made from a limesink pond near Florida, Puerto Rico, November 5, 1959. One of these snails was exposed on November 6, 1959, to *S. mansoni* miracidia from feces of a laboratory-infected white mouse. Miracidial penetration in the head region of the snail was observed. Twenty-two days later the snail released cercariae, which examination revealed to be stylet cercariae of a non-human fluke, i.e., not *S. mansoni*. The snail apparently was infected from a natural source prior to collection. However, 34 days after miracidial penetration, cercariae of *S. mansoni* were observed being shed by the same snail. They were shed for 5 days. The snail died on the 6th day, at which time it was 6.1 mm in diameter. This snail had been isolated throughout the experimental period and had laid eggs yielding progeny.

Two offspring from this snail were exposed to miracidia from feces of the previously used mouse on the following dates: December 24, 1959; April 2, 8 and 19, 1960; and May 5 and 22, 1960. After examinations at irregular intervals with negative results, both snails (5.5 and 5.2 mm in diameter) showed heavy infections with *S. mansoni* on June 2, 1960, with developing larval stages of parasites in the head, neck, and foot. The larger of the two snails was shedding cercariae June 2, 1960, and the smaller died without shedding. From the observed intervals, it appears probable that the first four exposures did not result in successful development of *S. mansoni*; but these exposures may have had some influence on the successful infection. —CHARLES S. RICHARDS, *Puerto Rico Field Station, Technology Branch, Communicable Disease Center, Public Health Service, U. S. Dept. of Health, Education and Welfare, San Juan, Puerto Rico.*

FURTHER STUDIES ON *TRANSVERSOTREMA LARUEI* VELASQUEZ WITH OBSERVATIONS ON THE LIFE CYCLE (DIGENEA: TRANSVERSOTREMATIDAE)*

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From November 1958 to January 1960 several hundred specimens of operculate snails, *Thiara riquettii* Grateloup** were collected at irregular intervals from fish ponds in the Experimental Station of the Philippine Bureau of Fisheries at Dagatdagatan, Malabon, Rizal Province, Luzon Island. Of the 701 snails examined for possible larval trematode infection, 96 harbored a transversotrematid cercaria which upon critical study proved to be that of *Transversotrema laruei* Velasquez, 1958.

Our present knowledge of the members of the family Transversotrematidae Yamaguti, 1953, is fragmentary. Soparkar (1924), Brien (1954), and Crusz (1956) reported the occurrence of *Cercaria patialensis* Soparkar in India, the Congo, and Ceylon respectively. *Cercaria koliensis* was found by Olivier (1947) in Guadalcanal, Solomon Islands. *Transversotrema haasi* Witenberg, 1944, from the Red Sea is the only adult ever described, but without host or organ record. *Transversotrema laruei,* a metacercaria was found by the author in the muscles and the intestine of a sea bass, *Lates calcarifer* Bloch, obtained from brackish water fish ponds at Malabon, Rizal Province.

Information on the cercaria and the early developmental stages in the snail host and the progenetic metacercaria is herein reported for *Transversotrema laruei.*

MATERIALS AND METHODS

To obtain the incidence of infection in the intermediate host in the area studied, the snails were crushed, dissected, and the early developmental stages recovered. Of the snails examined ranging from 1.8 to 24 mm long, those smaller than 8 mm were not infected. Several other gastropods in the area were also examined but were negative for the transversotrematid cercaria.

Naturally infected snails collected from the fish ponds were kept in finger bowls with a small amount of tap water and algae under laboratory conditions at room temperature of about 33 to 37 C. The emerged cercariae were collected by means of a pipette drawn to capillary

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Identification of the fish hosts were made by Mr. Agustin F. Umali, Ichthyologist, Philippine National Museum.

** Identified through the courtesy of Dr. Henry van der Schalie, Professor of Zoology, Curator of Mollusks, Museum of Zoology, University of Michigan, Ann Arbor, Michigan. Separate lots of the snail host were sent to Dr. van der Schalie and Dr. Harald A. Rehder, Curator of Mollusks, U. S. National Museum. Dr. Joseph P. E. Morrison, Associate Curator, U. S. National Museum, identified the snail as *Sermyla riquettii* (Grateloup). Dr. Rehder's letter came after the completion of the manuscript.

size. Both living and stained specimens were studied. The excretory system was observed in living cercariae. The early developmental stages, the rediae and cercariae, were fixed in hot formalin, stored in 70 percent alcohol, and stained in alcoholic cochineal. Some infected snails were fixed in hot formalin, sectioned 10 microns thick, and stained in Delafield's hematoxylin.

The Experimental Station at Dagatdagatan upon request kindly furnished the author at irregular intervals for a year with all available species of fish caught in the fish ponds except *Lates calcarifer*. Specimens of the latter species were bought from Malabon market. Although available most of the time, they are more abundant in November and December.

RESULTS

Out of about a dozen species of fish examined, *Lates calcarifer* and a mudfish (top minnow), *Mollienesia latipinna* Le Sueur, were more heavily infected with *Transversotrema laruei* than the spadefish, *Scatophagus argus* (Linnaeus); mullet, *Mugil sp.;* small tarpon, *Megalops cyprinoides* (Broussonet); *Tilapia mossambica* (Peters); short-finned gizzard shad, *Anodontostoma chacunda* (Ham.-Buch.); halfbeak, *Hemiramphus georgii* Cuvier and Valenciennes; and silver therapon, *Therapon argenteus* (Cuvier). Careful examination was made of all organs of every fish to determine the exact location of the parasite in question.

The worms were located under the scales and a few were found in the opercular cavity, gills, and in one instance in the muscle but none in the intestine. Five metacercariae with a single egg each were found under the scales of 2 out of 10 *Lates calcarifer*. The fish measured 1 to 2.5 feet in length and weighed 2 to 15 pounds. However, a single specimen with an egg was found in a single 2.5-inch mudfish (top minnow), *Mollienesia latipinna,* of 23 examined.

Mollienesia latipinna kept in aquaria with fresh water under laboratory conditions were successfully infected by releasing several normally emerged cercariae into the aquarium. About a month after infection, examination of the fish yielded only from the scales a metacercaria with well-developed gonads and vitellaria but no eggs. Due to lack of laboratory facilities no experimental infection was attempted with *Lates calcarifer*.

The metacercariae were fixed in Bouin's fluid under coverslip, stored in 70 percent alcohol, and subsequently stained as were the cercaria.

Measurements in millimeters, unless stated otherwise, were made of fixed specimens of rediae, 10 naturally emerged cercariae, and 6 metacercariae, each with a single egg.

Redia (Figs. 4 and 5)

Development of cercariae in rediae. Rediae oval, elongate and posteriorly attenuated, with well-developed pharynx and simple gut extending to about middle of body. Maximum length of redia not more than 1.0. Rediae containing 7 to 12 germ balls. Mother rediae containing daughter rediae but no cercariae. Digestive gland of infected snails presenting peculiar whitish appearance; upon dissection of the snail tissue in this area, various stages of developing cercariae were released, indicating that the germ balls develop outside the redia. Olivier (1947) reported a similar observation in *Cercaria koliensis.*

Cercaria (Figs. 1, 2, 3, 6, 7, 8, and 9)

Cercaria large, biocellate, furcocercous, protandrous and sexually mature. Body broader than long; length 0.28 to 0.43, average 0.35; width 0.48 to 0.7, average 0.59. Stem of tail 0.4 to 0.43 long, average 0.42; 0.09 to 0.12 wide, average 0.11. Furcae spatulate, thin, notched at distal ends; length 0.18 to 0.25, average 0.22; width 0.07 to 0.09, average 0.08. At base of tail a pair of bilaterally arranged appendages with adhesive pads at distal ends; length 0.12 to 0.18, average 0.15, width 0.03 to 0.05, average 0.04. Eye spots large, pigmented, just posterior to pharynx, at about level of acetabulum. Ventral surface of body covered with scale-like spines arranged diagonally (fig. 7); dorsal surface with minute spines, but hardly discernible.

Acetabulum pedunculate, disc-shaped, 0.09 to 0.12 in diameter, average 0.1; with sparsely scattered scale-like spines. Oral sucker absent, mouth opening subterminal, pharynx 0.05 in diameter. Esophagus elongate, narrow, bifurcating at level of acetabulum, uniting posteriorly to form cycloid intestine. When stretched, intestinal wall smooth; on contraction forming series of continuous small diverticula. Testes well developed, lobed, bilateral, intercecal, containing compact mass of active spermatozoa. Seminal vesicle dextral, also filled with moving spermatozoa; many being released to outside through conspicuous vas deferens leading to the genital pore at mid-anterior margin of body. Testes developed even in young cercariae with undeveloped lateral appendages (fig. 2). Ovary less developed than testes, smaller, sinistral; oviduct funnel-shaped, uterus fully formed, receptaculum seminis absent.

Excretory bladder (fig. 8) short, inflated anteriorly, receiving pair of extracecally placed ciliated siphons or collecting tubes, designated as primary, describing large loose loops in lateral areas of body. Junction of primary and secondary collecting tubes located posterior to intestine and testis. Secondary collecting tubes arising in anterior and lateral fields of body and receiving tertiary collecting tubes, one pair from tail. Two commissural vessels, one anterior to pharynx, other posterior to eye spots, connecting anterior secondary collecting tubes of two sides. Caudal excretory tube surrounded by large caudal bodies (fig. 6), continuing through tail stem, branches discharging to outside at indentations of spatulate furcae. Four pairs of flame cells in tail. Flame cells of body incompletely recorded.

Metacercaria (Fig. 10)

Specific diagnosis: Metacercaria progenetic; leaf-like, broader than long. Length 0.23 to 0.55, average 0.39; width 0.46 to 0.9, average 0.68. Eye spots present. Spination of body as in cercaria. Acetabulum essentially as in cercaria, 0.07 to 0.09 in diameter, average 0.08. Oral sucker absent. Mouth opening directly into pharynx, 0.04 to 0.05 in diameter, average 0.045. Esophagus narrow, bifurcating just anterior to midbody; branches uniting posteriorly forming cycloid intestine. Arrangement of male genital organs essentially as in cercaria. Female genital organs well developed. Ovary with indented margin, containing germinal follicles, sinistral, anterior to testis but smaller. Oviduct short, funnel-shaped. Oötype complex present; Laurer's canal prominent. Uterus well formed with transverse and ascending parts; transverse part passing over left testis, descending to inter-testicular level, then ascending and passing above the right testis to form ascending limb, latter discharging into common genital atrium. Receptaculum seminis uterinum present. Vitellaria follicular, heavily developed, extra-intestinal with wide anterior gap and interrupted posteriorly by excretory bladder. Vitelline reservoir transversely elongated, between acetabulum and transverse portion of uterus, formed by union of paired vitelline ducts (fig. 10). Eggs large, 125 to 150 microns long, average 137.5 microns by 46 to 81 microns wide, average 63.5 microns. In specimens examined, single yellow colored egg with miracidium occupies entire inter-testicular space (figs. 10 and 11).

DISCUSSION

Morphologically, the early developmental stages and the cercaria of *Transversotrema laruei* closely resemble those of *Cercaria patialensis* and *C. koliensis*. However, the excretory system of *T. laruei* is closer to that of *C. koliensis* except for the absence of the excretory commissure immediately above the excretory bladder in *T. laruei*. There is some indication of a commissure just posterior to the inflated portion of the bladder in my species. Moreover, *C. koliensis* has only one anterior excretory commissure. The cercaria of *T. laruei* is smaller than *C. patialensis* but bigger than *C. koliensis*.

My description (1958) of *Transversotrema laruei* was based on 3 metacercariae obtained from one *Lates calcarifer* of 10 examined; one was found in the muscle and two in the intestine. The fish were bought from the Central and Quiapo markets at Manila but had been caught from fish ponds at Malabon, Rizal Province. The practice of the fish vendors of making the fish seem fresh by continuously immersing and washing the fish in a tubful of water or in some cases under running water favors the loss of the ectoparasites especially those from the scales. The examination of newly caught, unwashed fish increased the possibility of getting worms alive and attached to the specific organ and host. However, a study of the

movements of the cercaria does not rule out the possibility that metacercariae may be found in the opercular cavity, gills, muscles, and intestine. The cercariae when resting assume a position as in figure 9. They may be carried with the water lodged in the mucoid substance of the gills and the opercular cavity, or perhaps swallowed. The worm may get into the muscle through a perforation in the scales along the lateral line system; scales of *Lates calcarifer* located along the lateral line system had such holes big enough to admit a cercariae.

Despite the considerable number of cercariae released in the pond water, this species exhibits a certain degree of host and organ specificity. *Thiara riquettii* is the only snail found infected although about six other species of snails exist in the pond. The progenetic metacercariae were obtained only in the fishes *Lates calcarifer* and *Mollienesia latipinna*, while *Scatophagus argus*, *Mugil* sp., *Megalops cyprinoides*, *Tilapia mossambica*, *Anodontostoma chacunda*, *Hemiramphus georgii* and *Therapon argenteus* may be infected with fewer mature metacercariae.

Progenesis has been reported in metacercariae encysted in arthropods (insects, crustaceans); also in fishes and amphibians and at times in molluscs, as cited by Buttner (1950) and Dawes (1946). The metacercaria of *Transversotrema laruei* assumes adult characteristics and produces eggs without encysting. The shape of the body of the worm, the scale-like spines on the ventral side of the body, and the pedunculate, spined acetabulum provide for secure anchorage and protection under the scale of the fish host.

Based on the morphology of the metacercariae, *Transversotrema laruei* and *T. haasi* are congeneric but differ as to size, shape of body, arrangement of vitellaria, relative proportion of pharynx and acetabulum, shape of spines, and character of testes. The egg of *T. laruei* is very much larger than that of *T. haasi* which is 90 by 70 microns.

After Witenberg (1944) created the genus *Transversotrema* with *T. haasi* as type and subsequently erected the subfamily Transversotreminae for *T. haasi*, Yamaguti (1953) established the family Transversotrematidae. LaRue (1957), aware of the lack of information on the life cycle of the group, proposed the suborder Azygiata to include the superfamily Azygioidea Skrj. and Guschanskaja, 1956, and superfamily Transversotrematoidea LaRue, 1957. The character of the cercaria fits in the classification at the suborder level.

On the basis of my study the diagnosis of the superfamily Transversotrematoidea LaRue (1957) is emended. For the sake of completeness, this emended diagnosis is combined with the family diagnosis given by Yamaguti (1953).

Transversotrematoidea LaRue, 1957, emended

Superfamily diagnosis. Cercariae brevifurcate; tail stem bearing pair of anterior lateral appendages; body leaf-like; oral sucker absent; pharyngeate; acetabulum pedunculate; eye spots pigmented; branches of gut fused posteriorly; genital pore anterior, submarginal, ventral, median; protandrous; metacercaria progenetic. "*Body transversely elongated, with ventral acetabulum.* Testes in acetabular zone. Cirrus pouch absent. Ovary in acetabular zone. Vitellaria follicular, extensive, mostly extracecal. Excretory vesicle tubular, with terminal pore."

SUMMARY

The pre-metacercarial stages of *Transversotrema laruei* develop in the digestive gland of the brackish water snail *Thiara riquettii* Grateloup. The cercariae develop in rediae; are biocellate, furcocercous, protandrous and sexually well developed;

may develop to progenetic metacercaria in *Lates calcarifer* Bloch and *Mollienesia latipinna* Le Sueur.

Morphological comparisons with *Cercaria koliensis* Olivier, 1947, and *Transversotrema haasi* Witenberg, 1944, are herein presented.

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Explanation of Plate 1

Unless otherwise stated, drawings were made with the aid of a camera lucida.

Figure 1. Cercaria of *T. laruei* showing the relative position of organs and spatulate furcae of tail. Ventral aspect.

Figure 2. Early cercarial stage showing relative development of testes and anterior lateral appendages and excretory system.

Figure 3. Young cercaria with testes and ovary developed.

Figures 4 and 5. Rediae.

Figure 6. Tail stem of the cercaria showing cellular organization and flame cell arrangement. Diagrammatic.

Figure 7. Scale-like spines of the cercaria showing arrangement on the ventral surface.

Figure 8. Body of the cercaria showing the nature of the excretory system and other organs. Diagrammatic, ventral view.

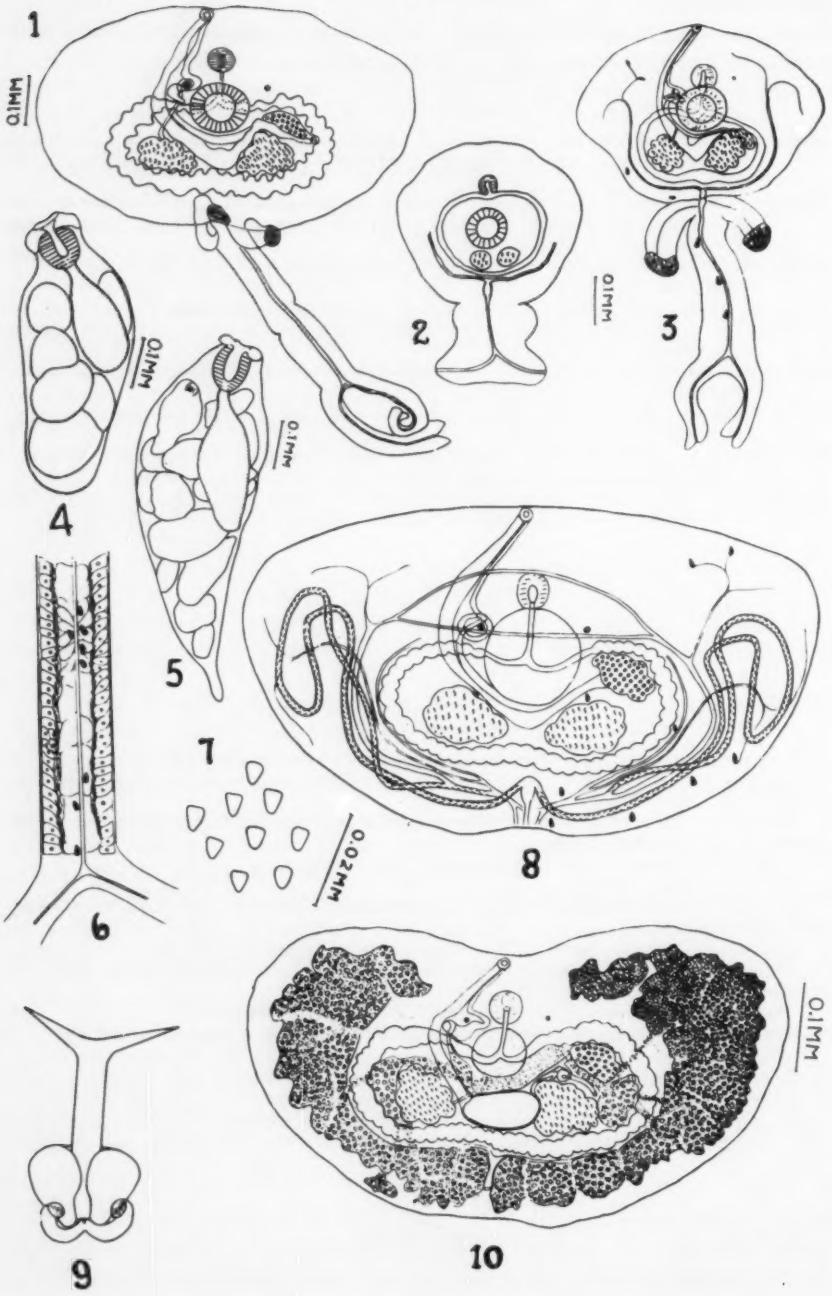
Figure 9. The cercaria in resting position in water. Diagrammatic.

Figure 10. Metacercaria showing form and relative position of organs, ventral view.

ERRATUM

Read aminitrozole wherever aminothiazol occurs in abstract 15, J. Parasit. **46** (supp.): 7–8.

PLATE I



STUDIES ON LARVAL *FASCIOLOIDES MAGNA*. I. OBSERVATIONS ON THE SURVIVAL OF REDIAE IN VITRO*

FRANK E. FRIEDL

Department of Zoology, University of Minnesota, Minneapolis**

Although some information is available concerning the in vitro development of trematode metacercariae (Ferguson, 1940, 1943; Smyth, 1959; Yokagawa et al, 1955), similar observations on intra-molluscan stages are scanty. Barlow (1925) reported the miracidium of *Fasciolopsis buski* underwent metamorphic changes followed by sporocyst growth and development in fluids expressed from snails and believed that parthenitae might be cultured in snail "lymph." Ingersoll (1956) observed survival times of rediae of *Cyclocoelum microstomum* in various complex media. These rediae, already present within miracidia in this species, were obtained suitable for culture by allowing the latter to swim free of microorganisms. Maximum survival of 14 days was achieved, "but no appreciable growth or development occurred."

The present preliminary studies on larval *F. magna* have provided much basic procedural knowledge and suggest certain interesting survival patterns under simple culture conditions.

MATERIALS AND METHODS

Each of the Ringer solutions shown in table I was used for dissecting infected snails and

TABLE I. *Composition of Ringer solutions M and C in grams per liter.*

	M	C
NaCl	7.00	6.50
KCl	0.25	0.14
$CaCl_2$	0.30	0.12
$NaHCO_3$	——	0.10
NaH_2PO_4	——	0.01

washing worms. Ringer M (table I) is a three-salt solution with standard KCl and $CaCl_2$ concentrations, but containing slightly less NaCl than usual; C is an amphibian Ringer with the bicarbonate content halved. These solutions were also employed in the survival experiments with test substances added as indicated.

Rediae of *F. magna* in various stages of development (ranging in size from about 0.5 mm to 3.0 mm in length) were dissected from laboratory-reared and infected *Lymnaea stagnalis jugularis*. Penicillin (250 units/ml) and streptomycin (250 micrograms/ml as the sulfate) were used for dissection and washing and in certain experimental media as shown. The worms were washed twice by changing the liquid in the 2-ml depression of a 75 by 45 by 6 mm tissue culture slide or by sedimenting through about 5 cm of fluid in 10 by 100 mm tubes. Washed rediae were then ready for the test media or saline controls used in 0.2-ml amounts in 2-ml pointed centrifuge tubes plugged with cotton, or later, rubber stoppers. Each tube received from 2 to 10 worms, transferred with the aid of a fine capillary pipette attached to a mouth tube. They were held at 20 C.

The criterion of survival was movement of the rediae. This corresponded well with their general appearance of viability. Observations were made daily. Despite the measures taken to

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render the worms free from bacteria, some tubes later showed contamination readily visible to the eye. No sterility tests with bacteriological media were performed.

Glassware for dissection and washing, and capillary pipettes for transferring were coated with silicone (General Electric SC-87, Dri-Film) which appreciably minimized the tendency of the worms to stick to their surfaces. Culture tubes were not siliconized. Sterile glassware and bacteriological techniques were used throughout.

When examining worms, distortion was reduced by inclining the tubes in water within a small plexiglass trough. A binocular dissecting microscope equipped with a 12× ocular and 6× objective was found satisfactory for viewing.

RESULTS

Salt solutions. Four physiological salt solutions had been previously tested, i.e. *Lymnaea* saline (Carriker, 1946) *Planaria* Locke (Murray, 1927), and Ringer M and C. The latter two performed as well or better than the others and were chosen for the study. Later, solution C proved more satisfactory and was used exclusively for succeeding experiments.

pH. Although hemolymph from the snail, *Lymnaea stagnalis jugularis,* has a pH of about 7.9 when freshly removed, rediae in vitro appear to tolerate the range of 7.0 to 8.0 in solution C. This saline tends to equilibrate with air to about pH 8.0, and in combination with most of the test substances gives solutions around neutrality.

Antibiotics. Penicillin and streptomycin (as the sulfate) when used together did not appear to markedly affect redial survival in solution C at concentrations under 800 units and micrograms per ml respectively. These antibiotics were used in experimental groups 1, 2, and 5.

Survival times. These are presented as bar diagrams in figures 1, 2, and 3. The time intervals represent the maximum number of days that tubes (usually two to a test) contained one or more moving rediae. Thus, a tube in which movement

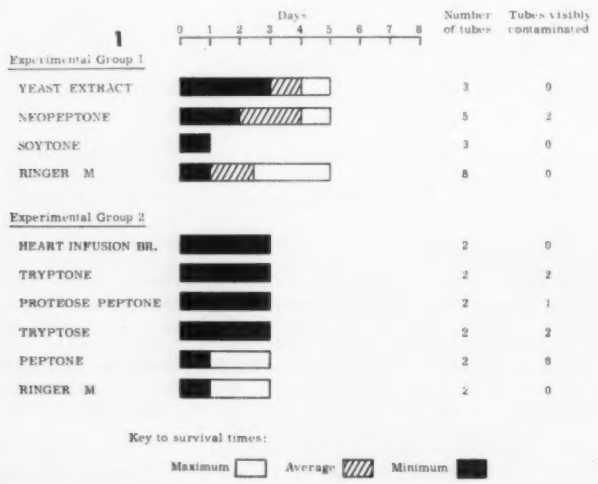

FIGURE 1. Survival of rediae of *F. magna* in 1 percent solutions of various dehydrated culture media (Difco) in Ringer M at 20 C. Penicillin (500 units per ml) and streptomycin (500 micrograms per ml as the sulfate) were included in all except proteose peptone and tryptose.

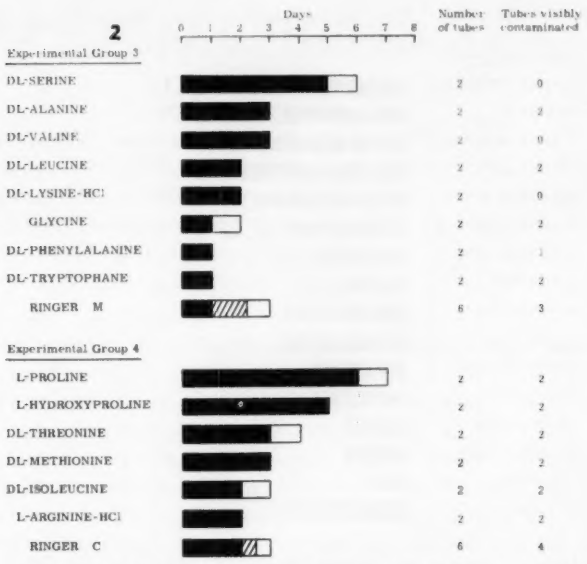

FIGURE 2. Survival of rediae of *F. magna* in 0.1 percent solutions of various amino acids in Ringer M and C without antibiotics at 20 C. Key as in figure 1.

ceased within the first 24 hours would appear as 1 day in the figure, one in which movement ceased between the 2d and 3d day would accordingly be recorded as 3 days. Where only two tubes are evaluated, the graph shows the maximum and minimum survival times, the code for minimum representing both if they gave identical results. Where three or more tubes are involved, the average longevity is included.

It might be mentioned that the results in some test solutions were more striking compared to their individual controls than is now apparent with the controls grouped and averaged. This is perhaps due to a variation in vitality of different lots of rediae.

Complex media. One percent solutions of various dehydrated media (Difco) containing denatured proteins and protein degradation products generally showed no marked extension of survival over controls (fig. 1). In group 1 yeast extract and neopeptone appeared more favorable than Ringer M in terms of average values, and in group 2 the bacteriological media gave survival approximating that of the controls. About 100 worms were used in these experiments.

Amino acids. Attention was next turned to survival times in 0.1 percent concentrations of various single amino acids in Ringer M or C. In figures 2 and 3 appear the results of studies with approximately 300 additional rediae in experimental groups 3 and 4 (without antibiotics) and 5 (with antibiotics).

Hydroxyproline, proline, and serine generally gave longer survival times than controls in all groups. In group 5, alanine, and the amino acid amide, asparagine, also appeared to exert some effect. Other amino acids, such as arginine, glycine, isoleucine, leucine, lysine, methionine, threonine, valine, and the amide, glutamine, seemed indifferent or did not prolong survival as noticeably relative to controls. Phenylalanine and tryptophane here appeared to shorten longevity.

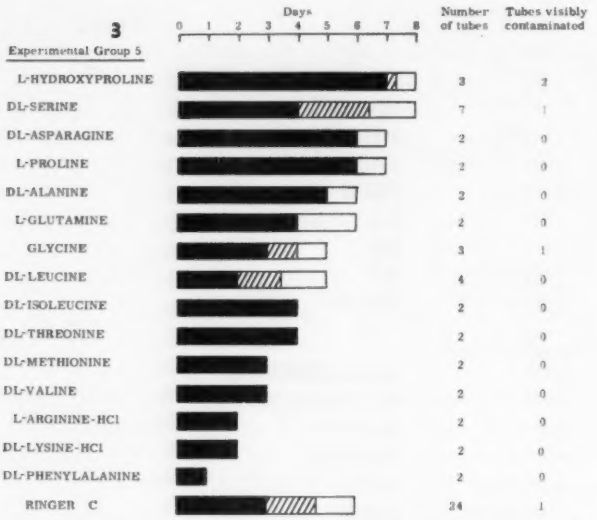

FIGURE 3. Survival of rediae of *F. magna* at 20 C in 0.1 percent solutions of various amino acids and the amides asparagine and glutamine in Ringer C containing antibiotics (penicillin, 250 units per ml; streptomycin, 250 micrograms per ml as the sulfate). Key as in figure 1.

DISCUSSION

The survival times obtained give some insight to the longevity of these worms under simple culture conditions. That no evidence of development was apparent within the periods under study is not surprising considering the composition of the test solutions. Even under apparently optimal natural conditions, Swales (1935), reported that *F. magna* required 49 to 58 days for cercarial production in a normal host, *Stagnicola palustris nuttalliana*. The value of the experimental "media" under study was therefore determined by survival time rather than by minute degrees of development, difficult to assess, except perhaps by histological methods.

Another factor bearing on the results is the possibility of damage being incurred to the worms during their removal, washing, and transfer to culture tubes. Because of this uncertainty, emphasis was placed on the maximum longevity obtained from a group of worms under similar conditions within the same culture tube.

Penicillin and streptomycin were found to be serviceable when employed prior to and within cultures. They may have considerable experimental value in culture studies, although Chernin (1957) and Chernin and Schork (1959) have noted that 100 micrograms per ml of streptomycin sulfate inhibit snail growth both in aquaria and in vitro.

Of the substances studied, the amino acids showed the most interesting results. It appears that a few of them were in some way prolonging survival in vitro. These amino acids are especially interesting for they provide a basis for speculation on relationships between larval trematodes and their snail hosts. The compounds in point, hydroxyproline, proline, and serine, are neutral amino acids, generally considered "non-essential" in higher animals, in which they are apparently derived

from carbohydrates and ammonia-yielding compounds. Here they seem to emerge as biochemically related substances of nutritive or protective value to larval *F. magna*.

SUMMARY

The survival of *F. magna* rediae, obtained from the snail *Lymnaea stagnalis jugularis*, has been studied in vitro in solutions containing individual amino acids, and in media containing denatured proteins and protein degradation products.

Of the substances tested, the most noticeable and consistent survival over that of Ringer controls occurred when the amino acids hydroxyproline, proline, and serine were present.

ACKNOWLEDGMENTS

The author wishes to thank Messrs. Walsh and Johnson of the Meat Inspection Division of the Minneapolis Department of Health and Dr. H. J. Griffiths of the College of Veterinary Medicine, University of Minnesota, for their assistance in obtaining the eggs of *Fascioloides magna* used in this study.

He is also indebted to Dr. F. G. Wallace of the Department of Zoology, University of Minnesota, for his aid and criticism during the work and to both Dr. Wallace and Dr. N. R. Stoll of The Rockefeller Institute, New York, for their comments on the manuscript.

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RESEARCH NOTE

THE SUSCEPTIBILITY OF THE CARDINAL, *RICHMONDENA CARDINALIS* (L.), TO *TRICHOMONAS GALLINAE* FROM A MOURNING DOVE, *ZENAIDURA MACROURA* (L.).

Many deaths of mourning doves (*Zenaidura macroura*) due to trichomoniasis have been reported by persons who maintain backyard feeding stations. For example, during the summers of 1958 and 1959, mourning dove deaths were reported at bird-feeding stations in Atlanta, Georgia; Arlington, Virginia; St. Louis, Missouri; and San Diego, California. Many persons who have feeding stations have expressed concern that other species may get the disease at the feeding areas.

This study was undertaken to determine whether the cardinal (*Richmondena cardinalis*) was susceptible to mourning dove isolates of *Trichomonas gallinae*. The cardinals were obtained by live-trapping on the Patuxent Wildlife Research Center, Laurel, Maryland.

Atlanta III isolate was obtained from a naturally-infected dove found dead at feeding trays maintained by William McBride at Atlanta, Georgia. An adult male cardinal was not infected in a preliminary experiment in which a culture of the Atlanta III isolate was placed in the drinking water. An immature mourning dove previously had been infected by this technique.

St. Louis I isolate of *Trichomonas gallinae* was obtained by George M. Senne from a naturally-infected mourning dove at Kirkwood, Missouri. An adult male cardinal was given 0.2 ml of St. Louis I culture, orally, on January 27, 1960. The bird remained normal until February 3, when it was killed for examination. The bird apparently was in good condition and had no obvious abnormalities. Internally, however, there was a thin serous exudate in the trachea, and both lungs were affected. The left lung had a 2 mm by 3 mm circular, raised caseous lesion on the pleural surface. Two smaller (less than 1 mm in diameter) yellow foci were nearby. The large yellow necrotic area was surrounded by a black hemorrhagic zone, 1 to 3 mm wide. The black zone separated the caseous lesion from the remaining lung tissue, which appeared normal. The lesions in the right lung were more extensive, involving the entire posterior portion of the lung with fibrinous adhesions between the lung and the costal pleura. Impression smears made of the caseous material showed large numbers of trichomonads and an unidentified microfilaria. No trichomonads were found in the oral cavity by direct smear or by culture using Diamond's medium (Diamond, L. S., 1957, J. Parasit., 43: 488–490).

A mass of caseous material was removed from the lung of the male cardinal, macerated, and placed in Diamond's media for 24 hours at 37.5 C. The following day, February 4, an adult female cardinal was exposed to infection by pipetting 0.2 ml of this culture into its mouth. On February 10 and 11 she was observed to be extremely depressed, her feathers were ruffled and she remained in one corner of the cage. She was found dead on the 12th. Autopsy showed that the right-posterior abdominal air sac was filled with a large yellow caseous mass. Microscopic examination of impressions of this air-sac material revealed numerous trichomonads.

A chunk of caseous material was removed from the female cardinal's air sac and fed to each of two male mourning doves that had been raised in the laboratory free of infection. Twelve days later one dove was found to have a typical trichomonad canker extending from the base of the tongue to the roof of the mouth and completely blocking the left side of the mouth. The second dove was observed to be acutely ill on February 25 and was found dead the next day. No canker was found in the mouth, but small, yellowish, necrotic areas were found in the crop. Cultures made from the oral cavity and from the crop were positive for trichomonads. The intestine was grossly inflamed, and death apparently was due to an enteritis of undetermined cause.

Appearance of caseous lesions in the lungs and air sacs of the cardinals is similar to cases reported by Levine et al (Am. J. Hyg. 33: 23–32, 1941). Their experimental English sparrows (*Passer domesticus*) developed lesions in the lungs and air sacs resulting, they believed, from aspiration of some of the inoculum. The cardinals were somewhat difficult to handle, and placing the inoculum in the mouth resulted in severe symptoms of difficult breathing. It is presumed that the cardinals aspirated some of the inoculum.

Although it can be concluded that cardinals are susceptible to infection with *T. gallinae*, in these studies cultures of *T. gallinae* had to be placed in the bird to produce infection. It is unlikely that cardinals could become infected at a feeding station unless a portion of a trichomonad canker were to become dislodged from the throat of an infected dove and subsequently be ingested by a cardinal.—LOUIS N. LOCKE, *Patuxent Wildlife Research Center, U. S. Fish and Wildlife Service, Laurel, Maryland.*

TWO NEW DIGENETIC TREMATODES FROM MARINE FISHES OF PUGET SOUND, WASHINGTON*

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The host fishes were collected during the months of July and August 1957, in the marine waters of the area of the San Juan Archipelago as a portion of a research program which was carried out by the author at the Friday Harbor Laboratories of the University of Washington.

Stafford established the genus *Fellodistomum* in 1904, using *Distomum incisum* Rudolphi, 1809, as the type species. The description was expanded and clarified by Nicoll in 1909, and verified by Miller in 1941 after a study of Stafford's report and trematode collection.

The description of the proposed new species is based on 101 specimens taken from the intestine of the lemon sole *Parophrys vetulus* Girard, 1854, from the areas listed below. Measurements for the holotype are given in millimeters, and, in parentheses, the range for the total number of specimens measured.

Fellodistomum phrissovum n. sp.

(Fig. 1)

Description (Based on 11 specimens): With the characteristics of the genus as described by Stafford, 1904 and expanded by Nicoll, 1909. Body flattened dorso-ventrally, elongate, tapering at ends; length of type specimen 1.270 (0.858 to 1.88) and maximum width at level of center of ventral sucker 0.742 (0.528 to 0.957). Oral sucker slightly subterminal, 0.189 in diameter (0.198 to 0.33). Oral opening antero-ventral in oral sucker. Ventral sucker 0.412 in diameter (0.297 to 0.577), in center of body. Prepharynx one-half diameter of oral sucker in length, joining oval, muscular pharynx at posterior border of oral sucker. Esophagus equal in length to pharynx, joins ceca at midline where they bifurcate, running posterolaterally to anterior edge of testes. Genital pore median or slightly left, anterior to ventral sucker in area of cecal bifurcation. Testes paired, ovoid, lateral, in posterior third of body, 0.181 by 0.313 (0.127 by 0.168 to 0.239 by 0.297). Cirrus sac elongate ovoid, median, anterior to ventral sucker, 0.346 in length (0.198 to 0.379). Ovary lobate, postero-lateral, between ventral sucker and right testis, 0.115 by 0.198 (0.066 by 0.132 to 0.148 by 0.264). Vitelline glands composed of discrete, but compactly placed vitelline follicles; lateral, extending from line of intestinal bifurcation to line of posterior half of ventral sucker. Uterus has several loops in posterior area of testes and one anteriorly directed loop, running dorsal to ventral sucker, to the genital pore. Excretory bladder V-shaped with terminal pore. Eggs spiny, 0.026 by 0.031 (0.019 to 0.037).

Host: *Parophrys vetulus* Girard, 1854, the lemon sole.
Location: Intestine.
Locality: East Sound of Orcas Island and Bellingham Bay, Washington.
Holotype: U. S. National Museum Helminthological Collection No. 59513.

Morphologically *Fellodistomum phrissovum* resembles both *F. agnotum* and *F. fellis* in general body shape; general distribution and type of vitellaria; length of the ceca; location of the ventral sucker, and location of the testes, ovary and

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genital pore. The new species differs from the closest related species, *F. agnotum* in several respects. The largest mature specimen of *F. phrissovum* measured 1.88 in length as compared to 3.3 for *F. agnotum;* prepharynx and esophagus present in *F. phrissovum* but not in *F. agnotum;* eggs of *F. phrissovum* 0.026 by 0.031 and spined, while those of *F. agnotum* were 0.024 by 0.048 and unspined; the testes and ovary in *F. phrissovum* nearly twice as large as those of *F. agnotum.*

The following species are herein accepted as valid: *Fellodistomum fellis, F. agnotum, F. furcigerum, F. magnum, F. profundum, F. sebastodis, F. preovericum, F. mendezi* and *F. phrissovum* n. sp.

Looss (1901) established the genus *Zoogonus,* for *Z. mirus* from *Labrus merula* at Trieste.

The description of the proposed new species is based on 168 specimens taken from the intestine of the several hosts from the areas listed below. Measurements are given in millimeters, first for the holotype and then, in parentheses, the range in the total number of specimens measured.

Zoogonus dextrocirrus n. sp.

(Fig. 2)

Description (Based on 10 specimens): With the characteristics of the genus as described by Looss in 1901. Body elongate, flattened dorso-ventrally, tapering at ends; length of type specimen 1.409 (0.924 to 1.427). Maximum width at level of gonopore and center of ventral sucker 0.462 (0.280 to 0.511). Oral sucker ventral, at anterior end of body 0.173 (0.149 to 0.206). Oral opening antero-ventral in oral sucker. Ventral sucker 0.190 (0.132 to 0.198) slightly anterior to center of body on midline. Prepharynx 0.132 (0.083 to 0.132) in length, joins heavy, muscular pharynx 0.149 (0.066 to 0.149) just posterior to the oral sucker. Esophagus 0.528 (0.314 to 0.528) in length, running posteriorly from pharynx along midline to beyond midbody where it bifurcates just posterior to the testes to form two short, sacculate ceca. Genital pore lateral, on right side of body at level of ventral sucker. Testes paired 0.099 by 0.132 (0.083 by 0.116 to 0.116 by 0.116), round to slightly irregular and located toward sides in midbody region between posterior border of ventral sucker and anterior border of ceca. A single vas efferens runs from the median edge of each testis to the posterior end of the cirrus sac where they join and enter the bipartite seminal vesicle. Cirrus sac 0.182 (0.149 to 0.231) elongate ovoid, on right side in area from midline to gonopore with posterior portion between right testis and ventral sucker, filled with large glandular cells surrounding cirrus and seminal vesicles. Ovary 0.099 by 0.099 (0.066 by 0.132 to 0.116 by 0.116) round to lobate, in midline area between ends of ceca. Vitellarium compact, about one-half size of ovary and located just posterior to that organ. Seminal receptacle about one-half size of vitellarium and located to right of that organ, posterior to ovary. Laurer's canal present. Uterus filled with miracidia in various stages of development within the egg membranes, with several loops in posterior part of body which extend forward to area of vitellarium. Terminal loop runs anteriorly between the testes, then between right testis and cirrus sac, looping over lateral portion of cirrus sac and joining a common atrium at the gonopore. Excretory bladder, a small, membranous sac, located in posterior body, terminating in a posterior terminal pore. Cuticle spined. In 15 of the 20 hosts, the parasites lacked cuticle and were therefore unspined.

Hosts: Lepidopsetta bilineata (Ayres), 1855, the rock sole; *Microstomus pacificus* (Lockington), 1879, the Dover sole; *Lycodopsis pacificus* (Collett), 1879, the black-bellied eel-pout; *Lycodes brevipes* Bean, 1890, the short-finned eel-pout; *Parophrys vetulus* Girard, 1854, the lemon sole; *Lumpenus anguillaris* (Pallas), 1811, the eel-blenny and *Isopsetta isolepis* (Lockington), 1880, the butter sole.

Location: Intestine.

Locality: East Sound and West Sound of Orcas Island and Bellingham Bay, Washington.

Holotype: U. S. National Museum Helminthological Collection No. 59514.

The new species is readily separated from other species of the genus by the dextral location of the cirrus sac and gonopore and is the first of its genus to be reported from the Eastern Pacific. It is further separated from the nearest related species *Z. pagrosomi,* in that the prepharynx is short and broad; esophagus is

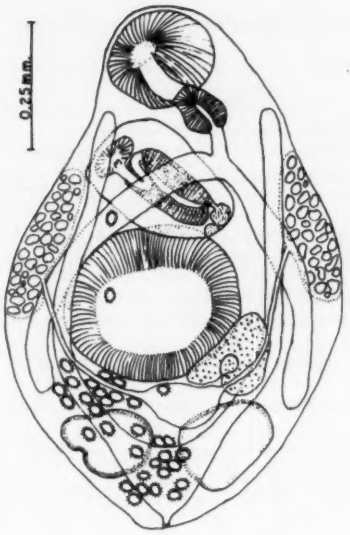

FIG. 1

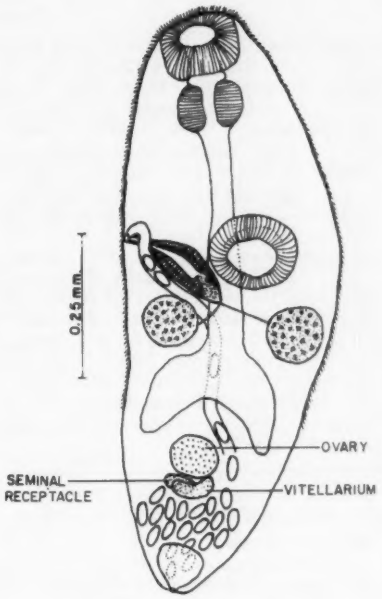

FIG. 2

FIGURE 1. *Fellodistomum phrissovum* n. sp. Adult, dorsal view.
FIGURE 2. *Zoogonus dextrocirrus* n. sp. Adult, ventral view.

broad; uterine coils entirely confined to body posterior to vitellarium and containing only single eggs in series; uterus not coiled anterior to vitellarium. These characters are in direct contrast to *Z. pagrosomi.*

Although Skrjabin (1957) made *Zoogonus mirus* Looss, 1901, a synonym of *Z. rubellus* (Olsson, 1868) Odhner, 1902, there seems to be insufficient evidence in the literature for doing so.

In the illustration by Looss (1901) of *Zoogonus mirus* n. g., n. sp., and in the illustration of *Z. rubellus* by Odhner (1902), both of which are labelled ventral view, the vasa efferentia go from the testes to the posterior end of the cirrus sac ventral to the esophagus and ceca. This is also the case in *Z. dextrocirrus.* On the assumption that this is a consistent anatomical characteristic of the genus, we must assume that the illustration of *Z. mirus* by Goldschmidt (1902) is a dorsal view, although he did not designate it as such in his paper. This would correlate the location of the cirrus sac and genital opening of Goldschmidt's specimens with their sinistral location in the type. The copy of Goldschmidt's illustration of *Z. mirus* by Stunkard (1938) is labelled "dorsal," which seems to be correct, but the drawing is incorrect since it shows a view which is partly ventral and partly dorsal. This is inconsistent with the original illustration. Stunkard (1938, 1940, 1941) plainly demonstrated that considerably more work must be done on life cycle and developmental stages before the Atlantic and Mediterranean species can be definitely placed in their taxonomic positions.

The recognized species are: *Zoogonus mirus, Z. rubellus, Z. pagrosomi* and *Z. dextrocirrus* n. sp.

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CHEMOTHERAPY OF HEXAMITIASIS IN FISH*

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Hexamita salmonis, the causative agent of hexamitiasis in salmonoid fishes, is endemic in most trout and salmon hatcheries throughout North America. The etiologic agent, a protozoan flagellate, ostensibly causes cellular damage in the caecal mucosa of afflicted fishes. It is also believed that heavy infections may interfere with normal growth by direct competition with the host for available nutrients in the intestinal tract. While the role of this supposed pathogen is relatively unclear, its presence in test fishes at this laboratory has caused considerable concern during the conduct of controlled nutritional studies.

Although McNeil et al (1941) showed that the incidence of *Hexamita* infections is widespread, hexamitiasis appears to be commercially important only in turkeys (Almquist and Johnson, 1951) and fish (Davis, 1953).

Very little has been reported on the protozoacidal effects of various drugs on the *Hexamita* infections in fish (Fish and McKernan, 1940; Smith and Quistorff, 1940; and Nelson, 1941). The most widely used chemotherapeutic agents are p-carbamidobenzene arsonic acid (carbarsone) and mild mercurous chloride (calomel). Initial attempts at this laboratory to control the parasite in infected fish populations using these two drugs at the recommended concentrations demonstrated that the former was erratic in effectiveness and the latter was toxic and produced loss in fish weight. The present study, therefore, was undertaken to find more effective therapeutic agents which would be palatable, non-toxic, and still effectively eradicate the protozoan from fish.

MATERIALS AND METHODS

Fingerling chinook salmon (*Oncorhynchus tshawytscha*), with a naturally occurring mild infection of *Hexamita salmonis,* were used throughout the study and fed the purified basic diet of DeLong et al (1958), in which the powdered chemotherapeutic agents (table I) had been uniformly blended. All test diets were stored at −10 C, daily portions being removed and refrigerated at 5 C until fed in the manner described by Halver (1957). The amount of diet presented to the test fish allowed maximum food intake by each fish without excessive wastage of food. To minimize reinfection, feeding implements were dipped in calcium hypochlorite solution for 10 to 15 minutes before use.

All diets were fed twice daily, morning and afternoon, for 5 days. On the sixth day, those groups of fish on therapeutic rations were returned to the basal diet until termination of the experiments. A daily record of mortalities was maintained. Each lot of fish was weighed at the beginning and end of the experimental period in order to obtain the average weight gain.

In the first or "screening" experiment, 150 fish from the 1958 hatch were placed in each test trough. These fish averaged 5.02 and 5.46 g for the 0.2 percent level and 1.0 percent level,

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respectively. Twenty-four compounds (table I) were tested for effectiveness at two therapeutic levels: 0.2 percent and 1.0 percent of the wet weight of the diet. The food presented to each group of fish was restricted to 20 g of test diet per day during the therapeutic period in order to insure complete consumption of all food offered. This amount corresponded to a therapeutic dose of 0.26 mg of drug per fish per day and 1.33 mg of drug per fish per day, respectively. During the recovery period, basal diet was fed ad libitum to all groups.

TABLE I. *Compounds* tested for hexamitacidal activity.*

Drug No.			
1	Erythromycin Stearate (56 percent)	Erythrocin Stearate	Abbott Laboratories
2	Fumagillin (94 percent)	.....	Abbott Laboratories
3	N,N'-Bis(2,2'dimethyl-6,6'diamino-4-quinolyl)-6-diaminohexane	.....	Abbott Laboratories
4	.....	PR-2581**	Abbott Laboratories
5	.....	PR-3714**	Abbott Laboratories
6	Acrizane-carbinol	.....	Abbott Laboratories
7	Chlorotetracycline hydrochloride (5.5 percent)	Aureomycin	American Cyanamid Co.
8	2-amino-5-nitrothiazole	Enheptin	American Cyanamid Co.
9	2-chloro-7-iodo-8-hydroxyquinoline	Vioform	Ciba Pharmaceutical Co.
10	4,7-phenanthroline-5,6-quinone	Entobex	Ciba Pharmaceutical Co.
11	Arthinol	Balarsen	Endo Products, Inc.
12	Diiodohydroxyquinoline	Yodoxin	Glenwood Laboratories, Inc.
13	p-carbamidophenyl arsenoxide	Carbarsone oxide	Eli Lilly and Co.
14	p-carbamidobenzene arsonic acid	Carbarsone	Eli Lilly and Co.
15	Glaucarubin	Glaumeba	Merck Sharp and Dohme
16	Nithiazide	Hepzide	Merck Sharp and Dohme
17	Mercurous chloride	calomel	Merck Sharp and Dohme
18	N,N'-dipiperonyl-5,11-diaminopentadecane-dihydrochloride	.....	Miles-Ames
19	5,11-Bis (3-methyl-6,7-dimethoxy-1,2,3,4,-tetrahydro 1-isoquinolyl) pentadecade dihydrochloride	.....	Miles-Ames
20	6,6'-diallyl-a a' bis (diethylamino)-4,4'-bi-o-cresol dihydrochloride	Camoform	Parke, Davis and Co.
21	2,2-dichloro-N-(2-hydroxyethyl)-N-(4-pyridylmethyl) acetamide	.....	Parke, Davis and Co.
22	Oxytetracycline (5.5 percent) hydrochloride	Terramycin	Chas. Pfizer and Co., Inc.
23	Diiodohydroxyquinoline	Diodoquin	G. D. Searle and Co.
24	2,3'dimethylquinoxaline-1,4-dioxide	.....	Smith Kline and French Laboratories

* The authors are grateful to the pharmaceutical companies listed for generous supplies of their respective compounds.
** Formula not available.

Some of the more promising compounds were also tested more extensively on smaller fish from the 1959 hatch. Because of limitations in experimental hatchery space, amount of available drug and quantities of infected fish, only eight compounds could thus be examined. These were tested at 0.01 percent, 0.04 percent, 0.2 percent or 1.0 percent levels using 60 fish (average weight 1.06 or 1.33 g) in each test group. Test diets in the second experiment were fed ad libitum and a record was maintained of the food consumed by each group. This food consumption corresponded to drug dosage of 0.01, 0.055, 0.275, and 1.4 mg of drug per fish per day, respectively.

The procedure used for the examination and rating of relative degrees of parasitic infection was similar to that reported by Fish and McKernan (1940). To minimize contamination, freshly sacrificed fish were initially submerged in 95 percent ethanol for approximately 15 seconds and then air-dried. An incision was made in the abdomen exposing the gut and the entire intestine was excised. The intestinal contents were expressed into a slide, mixed with a few drops of water, and examined under low power objective of a microscope (100×). Each slide was evaluated and assigned a number representing the relative degree of infection (footnote: table II). Five fish, selected at random from each trough, were examined as designated in tables II and III to provide the average degree of infection for each test group. Two samples of diiodohydroxyquinoline (Diodoquin and Yodoxin) were inadvertently included in experiment 1, thus providing (table II) a good indication of the reliability obtained in this method of testing drug efficacy.

RESULTS

Twenty-four compounds were evaluated for activity against hexamitiasis in chinook salmon fingerlings. The criteria of antihexamital effectiveness included severity of infection, fish mortalities, and relative weight gain. The data are summarized in table II.

TABLE II. *Experiment 1: Effect of drugs against* H. salmonis *infestation.*

Drug No.	Relative *H. salmonis* infestation*												0.2 percent level		1.0 percent level	
	0.2 percent level**					1.0 percent level***										
	Test period (days)			Recovery period (days)		Test period (days)			Recovery period (days)							
	3	4	5	8	16	2	3	4	5	6	9	12	Percent wt. gain†	Percent mortality†	Percent wt. gain	Percent mortality
13	0	0	0	0	0	0	0	0	0	0	0	0	17.3	0.7	−1.8	2.0
15	0	0	0	0	1	1	0	0	0	0	0	0	5.7	0.7	2.7	3.0
8	0	0	0	0	1	0	0	0	0	0	0	0	13.6	1.5	−4.9	3.0
5	0	0	0	0	1	0	0	0	0	0	0	0	14.1	2.7	1.3	2.0
16	0	0	0	0	3	0	1	1	0	0	0	1	3.8	5.3	2.5	1.0
2	3	0	0	0	0	3	2	0	0	0	0	0	16.3	2.0	6.6	4.0
17	0	1	0	0	1	0	0	0	0	0	0	0	5.4	0.7	−2.2	3.0
18	1	0	0	1	1	0	0	0	0	0	0	0	1.2	8.0	−4.7	16.0
11	1	1	0	0	1	1	1	0	0	0	0	0	14.9	4.7	−0.2	6.0
14	2	3	2	1	2	1	2	0	0	0	0	0	16.9	2.7	3.1	5.0
9	3	2	2	2	3	1	0	0	0	0	0	0	10.2	2.0	2.4	16.0
10	2	3	2	2	2	2	0	0	0	0	0	0	15.3	0.7	4.3	5.0
7	3	4	3	4	3	0	0	0	0	0	0	0	9.6	0.7	−2.0	4.0
19	1	3	3	1	1	0	1	0	0	1	0	1	7.9	6.0	2.4	4.0
1	3	3	3	4	3	2	2	1	0	0	1	1	10.3	2.0	2.2	1.0
4	1	2	2	2	2	1	2	2	1	1	1	3	6.1	2.0	−1.2	6.0
6	4	3	2	2	3	3	1	1	1	2	1	2	10.3	2.0	0.7	4.0
20	3	3	3	3	3	2	3	2	1	1	2	1	8.6	6.0	6.2	8.0
24	1	2	2	2	3	3	2	1	2	2	1	1	10.7	0.7	3.4	4.0
21	2	1	2	1	3	4	3	1	2	2	1	2	5.3	4.7	1.8	6.0
23	3	3	4	3	3	2	2	2	2	2	2	3	15.9	6.0	6.2	3.0
12	2	3	3	3	2	3	1	4	2	2	3	3	20.0	1.5	6.3	10.0
3	3	3	2	4	3	3	3	3	3	3	2	2	13.6	2.7	3.9	4.0
Untreated control	3	4	3	4	3	3	4	3	2	3	3	3	19.6	2.0	17.7	0

* Each value represents average of replicate counts from 5 fish.
0 = no hexamita on entire slide.
1 = 1–10 parasites per slide.
2 = 11–100 parasites per slide.
3 = 101–1000 parasites per slide.
4 = more than 1000 parasites per slide.
** 150 fish in each group, average initial weight 5.02 g.
*** 150 fish in each group, average initial weight 5.46 g.
† For 12 days.

At the 0.2 percent level p-carbamidophenyl arsenoxide, glaucarubin, 2-amino-5-nitrothiazole, PR-3714, nithiazide, and fumagillin all exhibited good activity against *Hexamita.* When the dose was increased to the 1.0 percent level, calomel, N,N′-dipiperonyl-5,11-diaminopentadecane-dihydrochloride, arthinol, p-carbamidobezene arsonic acid, 2-chloro-7-iodo-8-hydroxyquinoline, 4,7-phenanthroline-5,6-quinone and chlorotetracycline hydrochloride were also found to completely eliminate the parasitic infection. Unfortunately, N,N′dipiperonyl-5,11-diaminopentadecane-dihydrochloride and 2-chloro-7-iodo-8-hydroxyquinoline proved to be toxic to the experimental fish at this more effective dosage (table II).

Further studies carried out on smaller salmon confirmed the effectiveness of the drugs previously found to be most active (table III). In general, the maximum therapeutic response occurred at the same concentrations as in experiment 1. However, it is interesting to note that fumagillin appeared to be active at a much lower level.

A number of the drugs tested in this study interfered with growth of the experimental fish (table II). Thus glaucarubin and nithiazide, two of the most effective drugs at the 0.2 percent level, brought about a reduction of weight gain in the respective groups of fish. At the 1.0 percent concentration, however, all drugs tested appeared to reduce growth. When smaller salmon were used as the test animals, reduction in weight gain was observed only with PR-3714, calomel, and 4,7-phenanthroline-5,6-quinone.

The reduction in growth of fish undergoing therapy as compared to the untreated controls could have resulted from a reduced palatability of the test diets to the fish or an actual inhibition of growth by the drugs. In the second experiment with smaller fish, test diets were fed ad libitum and a careful record of daily food consumption was maintained. It was apparent upon examination of these records that no great reduction of food intake occurred in any of the groups undergoing drug therapy. Consequently, the observed reduction in weight gain (table III) must arise via an inhibition of growth by the chemotherapeutic agents.

TABLE III. *Experiment 2: Effect of drugs against* H. salmonis *infestation.*

Drug No.	Level (percent)	Relative *H. salmonis* infestation*** Test period (days) 1	2	3	4	5	Recovery period (days) 8	9	10	12	Percent weight gain	Percent mortality
13*	0.01	3	2	2	2	2	1	—	0	3	20.8	6.7
13	0.04	2	1	1	1	1	0	—	0	1	12.5	0
13	0.2	0	0	0	0	0	0	—	0	0	22.8	1.7
13	1.0	0	0	0	0	0	0	—	0	1	24.8	1.7
8**	0.01	3	3	2	2	2	—	1	—	2	25.0	1.7
8	0.04	1	0	1	0	2	—	2	—	2	29.2	5.0
8	0.2	1	1	1	0	0	—	1	—	0	17.0	1.7
8	1.0	0	0	0	0	0	—	0	—	0	20.1	3.3
5**	0.01	4	4	4	3	3	—	2	—	2	20.3	0
5	0.04	4	2	2	2	0	—	1	—	2	13.0	6.7
5	0.2	1	0	0	0	0	—	0	—	0	8.5	0
2*	0.01	4	3	3	1	0	0	—	0	0	23.5	5.0
2	0.04	3	0	1	0	0	0	—	0	1	20.0	0
2	0.2	4	2	1	0	0	0	—	0	0	32.0	8.3
2	1.0	2	0	1	0	0	0	—	0	0	21.0	5.0
17*	0.01	4	4	3	3	4	2	—	3	4	11.5	0
17	0.04	3	3	2	3	1	1	—	3	3	12.7	0
17	0.2	3	1	2	0	1	0	—	0	0	3.8	1.7
10**	0.01	3	4	3	3	2	—	2	—	2	17.3	5.0
10	0.04	2	4	1	3	3	—	1	—	0	25.2	1.7
10	0.2	3	4	1	1	2	—	2	—	2	21.9	5.0
10	1.0	1	1	0	0	1	—	0	—	0	12.9	3.3
7**	0.01	4	4	3	2	4	—	3	—	3	18.1	8.3
7	0.04	3	4	4	3	3	—	3	—	2	15.1	1.7
7	0.2	4	4	3	2	3	—	3	—	3	19.5	1.7
14*	0.01	3	3	3	3	3	1	—	3	3	26.7	3.3
14	0.04	3	3	4	4	4	1	—	3	3	14.1	1.7
14	0.2	3	3	4	4	3	2	—	3	2	30.1	0
Untreated Control*		3	3	4	4	4	2	—	4	4	35.0	3.3
Untreated Control**		3	4	3	2	4	—	4	—	3	16.9	5.0

* 60 fish per trough, average initial weight 1.06 g.
** 60 fish per trough, average initial weight 1.32 g.
*** Each value represents average of replicate counts from 5 fish.

DISCUSSION

The possible pathogenicity of *Hexamita salmonis* in fish culture and nutrition studies has prompted a thorough search for effective chemotherapeutic agents. A mild *Hexamita* infection in chinook salmon fingerlings with resulting low mortalities provided an ideal system for screening such compounds. Drug efficacy, fish growth and drug toxicity could all be evaluated simultaneously under these conditions.

In the present studies a number of drugs have been found to be effective in eliminating *Hexamita salmonis* infections in the test fish. Although somewhat erratic in effectiveness, fumagillin eventually became active when supplied to the infected fish at a concentration of 0.04 percent in the diet. P-carbamidophenyl

arsenoxide was immediately effective at the 0.2 percent level and glaucarubin, 2-amino-5-nitrothiazole, PR-3714, and nithiazide became active after several days at this concentration. Calomel, N,N′-dipiperonyl-5,11-diaminopentadecane-dihydrochloride, arthinol, p-carbamidobenzene arsonic acid, 2 chloro-7-iodo-8-hydroxyquinoline, 4,7-phenanthroline-5,6-quinone and chlorotetracycline hydrochloride proved effective only at the 1.0 percent level. The observation that p-carbamidobenzene arsonic acid was effective only at a 1.0 percent concentration in salmon is in contrast to the results obtained for trout (Fish and McKernan, 1941; and Nelson, 1941). The therapeutic dose for those compounds effective in eliminating this parasite from fish (53 mg per kg per day and 254 mg per kg per day for experiment 1, and 10 mg, 41 mg, 206 mg and 1030 mg per kg per day for experiment 2) is much greater on a weight basis than that required to eliminate parasitic infections in humans. The optimum therapeutic dose required for such infections however, appears to vary widely according to size or species (Thompson, 1955), or both.

Drug toxicities in the form of fish mortalities or depressions in weight gain were observed with a number of the compounds tested. It is an important commercial consideration that fish should maintain or increase their growth rate while undergoing antihexamital therapy. The demonstration that certain drugs are effective in combating these infections, but interfere with normal salmon growth, could discourage use of such agents.

N,N′dipiperonyl-5,11-diaminopentadecane-dihydrochloride and 2-chloro-7-iodo-8-hydroxyquinoline produced excessive fish mortalities when included in diets at concentrations of 1.0 percent. Toxicities in the form of inhibition of growth were exhibited by a number of the drugs tested. Although the reason for the comparatively low percent weight gains of 1 percent level groups in experiment 1 (table II) is not clear, the data did correlate significantly with those observed in other groups. Glaucarubin and nithiazide, while effective in eliminating the parasite when fed at a concentration of 0.2 percent, definitely appeared to reduce normal fish growth. There was some indication that PR-3714 also inhibited growth at this concentration with younger fish. Similarly, calomel and N,N′-dipiperonyl-5,11-diaminopentadecane-dihydrochloride interfere with fish growth at the 0.2 percent level and require a concentration of 1.0 percent for therapeutic effect. It is interesting to note that calomel, which has been frequently used in treating *Hexamita* infections in fish (Davis, 1953), markedly decreases fish growth even at a concentration of 0.04 percent. McNeil and Hinshaw (1945) have also found that calomel, used to treat infections of *Hexamita meleagridis* in turkey poults, is non-toxic only at concentrations low enough to have no therapeutic value. At higher, more effective concentrations, it produces increased turkey mortalities and pronounced inhibition of growth.

SUMMARY

1. Twenty-four chemotherapeutic agents have been tested for hexamitacidal activity on *Hexamita salmonis* infections in chinook salmon fingerlings. When supplied at a concentration of 0.2 percent in the diet of the infected fish, p-carbamidophenyl arsenoxide, 2-amino-5-nitrothiazole, PR-3714, or fumagillin were effective in eliminating the parasite without apparent toxic effects to the host. At a 1.0 percent concentration, chlorotetracycline hydrochloride, arthinol, p-carbami-

dobenzene arsonic acid and 4,7-phenanthroline-5,6-quinone were also active.

2. Calomel, which has been used in the past for treatment of *Hexamita salmonis* infections, inhibits fish growth at the dosage necessary for therapy. P-carbamidobenzene arsonic acid (carbarsone) was not effective in salmon at the 0.2 percent concentration previously employed for similar infections in trout.

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A NOTE ON THE INCIDENCE OF THREE SPECIES OF *GIARDIA* IN MINNESOTA*

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Review of the literature does not give much information in regard to the incidence of *Giardia canis*. Gordon and Young (1922) reported 6.0 percent of the 50 dogs examined in Brazil were positive for *Giardia* cysts. Catcott (1946) reported an incidence of 17.7 percent, 11.5 percent cysts and 6.1 percent trophozoites, on a total of 113 dogs checked in Ohio. Choquette and Gelinas (1950), in their survey of intestinal nematodes and protozoa in dogs from the Montreal area, stated that they found 14.7 percent of the 68 protozoan positives to be *G. canis*. Actually, there were 14 positives for *Giardia* in a total of 155 stools, or an incidence of 9.0 percent. They used zinc sulfate flotation and did not mention observation of the trophozoite form. Craige (1948) reported 15 percent incidence in 160 dogs and stated that he used a "flotation technique," but did not name the one employed.

The literature on the incidence of other species of the genus *Giardia* is also very limited, with the exception of the human species, *G. lamblia*. There are numerous reports involving a wide range of percentages. They range from a 0.3 percent incidence reported by Smithies (1926) to 17.95 percent in school children reported by Stiles (1915). Highby (1930) reported a 9.0 percent incidence from 99 children examined in Minneapolis hospitals. Most of the high incidence figures are seen in data derived from the examination of children. The figures obtained upon examination of a mixed-age group are somewhat lower. Boeck and Stiles (1923) examined 13,043 stools and noted a 6.5 percent incidence in this group. The latest figure, quoted from Belding (1958), gives 7.2 percent as the incidence of *G. lamblia* in man.

METHODS

During the past 2 years data have been collected at the University of Minnesota on the incidence of *G. canis* in this area. The information was taken from the results of the routine fecal examinations made on dogs examined at the Veterinary Clinic. Examinations were made by using a zinc sulfate flotation technique; direct smears supplemented this examination when the stools were of fluid consistency. The direct smears were done to aid in the demonstration of trophozoite forms present, since this form does not usually rise to the surface of the salt solution.

RESULTS

A total of 2,063 dog stools were examined, 1,133 in 1958 and 930 in 1959. The parasite was found in 86 or 7.58 percent of the animals in 1958. One fecal sample contained motile trophozoites, while all of the other samples contained cysts. In 1959, 72 of the 930 dogs, or 7.74 percent, were positive for *G. canis*. Once again only one sample showed trophozoites. The average incidence over the 2-year period was 7.66 percent.

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Another interesting observation was the ages of the infected dogs. *G. lamblia* is considered a cause of disease of children. *G. canis* is also a parasite of young animals. The data collected in 1958 showed that 69.5 percent of the infected dogs were 6 months or less in age and 93.03 percent were less than 1 year old. In 1959 the results were very similar. Of the infected dogs examined, 70.1 percent were 6 months of age or younger and 85.1 percent were less than 12 months old. The figures showed no apparent correlation between the breed or the sex of the dogs in relation to infection with this parasite.

Positive fecal examinations from dogs were not observed consistently throughout the year. Several positive samples appeared on the same, or on successive days, then there was an extended period when *G. canis* could not be demonstrated. This periodic appearance and disappearance of the parasite has not been explained. Perhaps it was simply chance occurrence.

The information on the incidence of *G. cati* from the domestic cat was more limited, as far fewer stools from cats are examined. One hundred and eighty-eight were checked in 1958 and 3.2 percent of them were *Giardia*-positive. In 1959, 2.9 percent of the 103 animals examined were positive. This was an average of 3.05 percent for the 291 cat fecal samples examined over the 2-year period.

Wild mice (*Mus musculus*) were trapped from several buildings in the immediate area. The small intestine was examined for the presence of the trophozoite form of *G. muris*. Fourteen percent of the 57 mice examined were found to be infected. Most of these were young animals. Forty-eight of the mice weighed less than 20 grams, which indicates that they were probably less than 6 weeks old.

Our laboratory mouse colony is heavily infected with *G. muris*. The parasite has been observed in approximately 50 percent of the animals. This figure is rather high, but as this inbred colony contains cages of experimentally infected animals and since no attempt is made to control the transmission of the parasite throughout the colony, the high figure is understandable. Also, once again all the animals examined were young. A similar high incidence of *Giardia* in an experimental colony of hamsters at the Pasteur Institute in Paris was reported by Lamy and Mossion (1954).

DISCUSSION

The figures presented in this paper, at least for the canine cases, have been derived from a large enough population to be of significance. The data derived from the cats and mice are sufficient to give some indication of the chance of finding the parasite. The canine average percentage of 7.66 percent is markedly similar to the 7.2 percent compiled by Belding (1958) on the incidence of *G. lamblia* in humans.

SUMMARY

1. Examination of fecal samples from dogs, performed at the University of Minnesota Clinical Parasitology Laboratory of the College of Veterinary Medicine indicated that 7.66 percent of the 2,063 samples examined were positive for *Giardia canis*.

2. Three and five-hundredths percent of 291 cat fecal samples were positive for *Giardia cati*.

3. Fourteen percent of wild *Mus musculus*, from inside local buildings, were positive for *Giardia muris*. About one-half of the animals examined from our white

mouse colony in the laboratory were found to be infected with this parasite.

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RESEARCH NOTE

PARASITES OF IDAHO AMPHIBIANS.

During the summers of 1957 and 1958, one hundred and sixty-seven amphibians representing 14 different species were collected in Idaho for the purpose of parasitological examination.

The animals examined follow: 10 *Hyla regilla,* 13 *Rana pipiens,* 14 *Rana catesbeiana,* 46 *Rana pretiosa,* 19 *Rana pretiosa* × *sylvatica* crosses, 8 *Bufo boreas,* 2 *Bufo woodhousei,* 5 *Scaphiopus hammondi,* 4 *Ascaphus truei,* 3 *Taricha granulosa,* 1 *Ambystoma tigrinum,* 30 *Ambystoma macrodactylum,* 6 *Dicamptodon ensatus,* and 34 *Plethodon vandykei.*

Eight species of trematodes, 3 species of nematodes, and 1 species of cestode were found.

TREMATODA

Haematoloechus coloradensis (Cort, 1915), in the lungs of 2 *Rana pipiens.*

Haematoloechus longiplexus Stafford, 1902, in the lungs of 7 *Rana catesbeiana.*

Haematoloechus medioplexus Stafford, 1902, in the lungs of 8 *Rana pipiens.*

Haematoloechus parviplexus (Irwin, 1929), in the lungs of 1 *Rana pretiosa* and 13 *Rana pretiosa* × *sylvatica* crosses.

Haplometrana intestinalis Lucker, 1931, in the intestines of 31 *Rana pretiosa,* 9 *Rana pretiosa* × *sylvatica* crosses, and 1 *Bufo boreas.*

Gorgoderina translucida (Stafford, 1902), in the urinary bladders of 9 *Rana pretiosa,* 9 *Rana pretiosa* × *sylvatica* crosses and 1 *Bufo boreas.*

Brachycoelium salamandrae (Froelich) Dujardin, 1845, in the intestines of 4 *Ambystoma macrodactylum.*

Telorchis bonnerensis Waitz (in press), in the intestines of 3 larval *Ambystoma macrodactylum.*

NEMATODA

Aplectana gigantica Olsen, 1938, in the intestines of 8 *Rana pretiosa* and 8 *Rana pretiosa* × *sylvatica* crosses.

Oxyuris sp., in the large intestine of 12 *Ambystoma macrodactylum.*

CESTODA

Baerietta sp., in the intestines of 10 *Plethodon vandykei.*

Hyla regilla, Bufo woodhousei, Scaphiopus hammondi, Ascaphus truei, Taricha granulosa, and *Ambystoma tigrinum* were not found infected with any parasitic helminths.

This investigation was supported in part by a research grant from the Northwest Scientific Association and was completed under the tenure of a fellowship (EF-9587) from the U. S. Public Health Service.—J. ALLAN WAITZ, *Department of Zoology, University of Illinois, Urbana, Illinois.*

RESEARCH NOTE

INOCULATION OF HUMAN MALARIA INTO A SIMIAN HOST, *MACACA MULATTA*.

With the renewed interest in the relationships between man, monkeys, and their malaria parasites (Eyles, D. E., Coatney, G. R. and Getz, M. E. 1960, Science **131**: 1812–1813), several attempts at transmission of human malaria to simian hosts seem worth reporting. Subsequent to studies of the pre-erythrocytic stages of human malaria (Jeffery, G. M., Wolcott, G. B., Young, M. D. and Williams, D., Jr., 1952, Am. J. Trop. Med. Hyg. **1**: 917–926), three experiments were conducted where rhesus monkeys (*Macaca mulatta*) were inoculated with malarial parasites in heavy dosages. The first monkey, a 4-pound male, was inoculated with human blood containing *Plasmodium ovale* (Donaldson strain). About 52 million parasites were given intramuscularly, and 7 days later about 80 million parasites intravenously. No apparent parasitemia developed during the subsequent 8-week period of observation. This monkey then received whole salivary glands intravenously from 153 *Anopheles albimanus*, of which approximately 92 percent were very heavily infected with sporozoites of *P. falciparum* (Panama strain). On the fourth day after inoculation a sample of liver tissue was taken at laparotomy; at 6 days the monkey was sacrificed and samples of liver, lung, kidney, spleen, and sternal marrow fixed in Carnoy and Zenker fixatives. The second monkey, a 3.5-pound male, received whole salivary glands intravenously from 119 *A. quadrimaculatus*, of which approximately 56 percent were heavily infected with sporozoites of *P. vivax* (Chesson strain). In addition, approximately 150 heavily infected mosquito bites were administered on the same day. Six days later the monkey was sacrificed and samples of liver, lung, kidney, spleen, and sternal marrow fixed in Carnoy and Zenker fixatives. At the time of sacrifice thick blood films were prepared from both monkeys; no erythrocytic parasites could be found. There were no human controls to demonstrate the infectivity of sporozoites from the actual lots of mosquitoes used. However, with the strains of parasites and mosquito vectors used, in several hundred trials there has never been a failure in a non-immune human recipient. Methods used were identical to those where human subjects were successfully inoculated with massive sporozoite dosages.

After fixation, dehydration, embedding, and sectioning at 6 microns, tissue sections were stained with a colophonium-Giemsa method and examined microscopically for exo-erythrocytic forms. No such forms were found after careful search. Thus it appears that a pre-erythrocytic cycle of these parasite strains may not develop in non-splenectomized rhesus monkeys, even after what must be considered a massive inoculation. The monkeys had no known previous malarial history. It does not seem likely that the prior unsuccessful exposure of our monkey to *P. ovale* would have any significant effect on the subsequent *P. falciparum* inoculation. The failure of *P. ovale* to infect confirms the finding of Christophers (1934, Proc. Roy. Soc. Med. **27**: 991–1000), who similarly attempted infection of rhesus monkeys with *P. ovale*.

The absence of exo-erythrocytic forms of human malaria in monkeys may be of particular significance in view of the more recent work of Bray (1957. Am. J. Trop. Med. Hyg. **6**: 514–520; 1958, Ibid. **7**: 20–24) who found such forms in the liver of non-splenectomized chimpanzees after inoculation with sporozoites of *P. falciparum*, and which were presumed to exist in the case of *P. vivax*. Unless the chimpanzee had been splenectomized, no apparent erythrocytic cycle persisted. The refractiveness of various monkeys, including *M. mulatta*, to erythrocytic infection with *P. falciparum* and *P. vivax* has been demonstrated a number of times. Aberle, 1945, in her monograph on primate malaria (Natl. Res. Council, Div. Med. Sci. publication), reviewed much of the literature in this field. A number of investigators have inoculated monkeys (*M. mulatta*) with the sporozoites of *P. vivax* and have found no exo-erythrocytic forms (Garnham, P. C. C. and Bray, R. S., 1956, Rev. Bras. de Malariol. **8**: 151–159; Hawking, F. and Thurston, J. P., 1952, Tr. Roy. Soc. Trop. Med Hyg. **46**: 293–300; Huff, C. G. and Coulston, F., 1948, J. Parasit. **34**: 264–274).

The limited observations here reported suggest that in *M. mulatta* the liver and other internal organs do not support such exo-erythrocytic parasites of *P. vivax* or *P. falciparum*.—GEOFFREY M. JEFFERY, *Laboratory of Parasite Chemotherapy, National Institute of Allergy and Infectious Diseases, P. O. Box 717, Columbia, South Carolina.*

LIFE HISTORIES OF TICKS UNDER LABORATORY CONDITIONS (ACARINA: IXODIDAE AND ARGASIDAE)*

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Numerous investigations in recent years have been concerned with feeding blood-sucking arthropods through animal membranes. Tarshis (1958a, 1958b) reviewed the pertinent literature in this field and described a successful technique applicable to feeding *Ornithodoros* species. Whether or not all species of argasids will feed through membranes remains to be tested. Also, ixodids and other arthropods which require several days of feeding on a host animal have not been fed successfully by using the membrane technique.

The use of laboratory animals as hosts for blood-sucking arthropods still remains a time-proven method. Most colonization methods used in the past involved laborious animal work and host-feeding apparatus. At the same time, such methods did not provide exact data on the age of different stages of ticks. For these reasons, modifications and changes of rearing techniques were necessary in order to obtain ticks of known age for tests of insecticide toxicity. This paper describes some improved techniques which are relatively inexpensive and convenient to the time schedule of the scientific worker. Minimum time for life cycles of ticks reared under laboratory conditions are presented.

MATERIALS AND METHODS

Tick colonies of the following six species were established from material originating as indicated; *Argas persicus* (Oken), California; *Ornithodoros coriaceus* Koch, California; *Ornithodoros moubata* (Murray), Johannesburg, South Africa; *Otobius megnini* (Dugès), California; *Amblyomma americanum* (Linnaeus), College Station, Texas, and Fayetteville, Arkansas; *Dermacentor andersoni* Stiles, Rocky Mountain Laboratory, Hamilton, Montana.

A. persicus: The ticks were held in cotton-stoppered, 100-ml erlenmeyer flasks provided with strips of absorbent paper, since this species is positive thigmotropic. Except where otherwise indicated, this species was kept at a mean temerature of 25.7 degrees C (range, 23 to 26.5) in a cabinet in which the relative humidity ranged from 72 to 80 percent. Larvae, nymphs, and adults were fed on White Leghorn chickens following the procedure used by Loomis (1953). The bird was restrained and ticks were placed directly on the body below the wings. The bird's wings were restrained and the bird was placed in a large crock, the bottom of which was lined with paper to absorb the bird's feces (plate I, fig. 2). The top of the crock was covered with black muslin laid atop a circular wire screen. Nymphs and adults were removed from the layers of paper in the bottom of the crock. Only rarely were they forcibly removed with forceps from the bird at the end of 1- to 2-hours' exposure. For larvae, the bird was kept for 12 hours in the crock after which all bonds were removed and the bird maintained for 4 to 6 days in a 5-gallon metal cage. Black muslin over a square wire screen was placed on top of the cage and the entire unit placed in a large dishpan (plate II, fig. 3). This unit was then placed on a tick-barrier table similar to the "ticktorium" table used by Schuhardt (1940) for colonizing *Ornithodoros turicata* Dugès. This wooden table was 5 feet long by 2 feet 8 inches wide and 2 feet

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6 inches high (plate I, fig. 1). An aluminum, right-angled, J-shaped moat was placed around the table edges. The inner wall of the moat was fixed to the edges of the table so that the top 1 inch was above the table surface. Number 10 weight motor oil was added to the moat. Copper wire enclosed in 0.25-inch glass tubing was placed around the bottom inside edge of the inner wall of the moat. The wire was connected to a 110-volt, alternating current outlet at one corner of the table. These barriers successfully prevented the escape of ticks. After 4 days most of the engorged larvae were found between the layers of black muslin. Only a few engorged larvae had to be removed with forceps from the bird by the sixth day after attachment.

O. megnini: Since the immature stages of this species are entirely parasitic and the adults do not feed, a simplified and accelerated rearing technique was developed. Ear bags and cages applied to large and small domestic animals often resulted in physical discomfort to the host, damage by the ticks to the host's ears, repeated damage to the appliances, and loss of specimens (Hunter and Hooker, 1907; Herms, 1917; Woke, 1951). Larvae and nymphs were successfully fed on the closely shaved abdomen of domestic rabbits after the method of Kohls (personal communication) with guinea pigs. The rabbit was inoculated subcutaneously with a 60 mg per ml solution of Nembutal Sodium (0.5 ml per pound body weight) 0.5 hour prior to application of the ticks. The rabbit's legs were tied to opposite ends of a holding board 10 inches wide by 3 feet long. A rectangular cloth girdle, made of pillow ticking 8 inches long and 4 inches wide containing a 6-inch zipper sewn into place in the middle, was applied with adhesive tape to the shaved mid-body region of a rabbit (plate II, fig. 1). Larvae were applied by inverting the holding vial onto the skin until nearly all were attached. The zipper was closed and the rabbit remained on the board for about 0.5 hour before it was returned to a cage. Due to the detachment of the first-stage nymphs and the sloughing of skin around the sites of attachment, it was necessary to prepare a second animal and transfer the nymphs. The eggs and adults were stored in 50 ml flasks in the same cabinet used for *A. persicus.*

O. coriaceus: Adults and subsequent progeny were divided into three groups and each maintained under different conditions for 1 year. Two groups were stored in temperature cabinets, unless otherwise indicated, set at mean temperatures of 32.4 and 25.7 C (range, 31.5 to 33 and 23 to 27, respectively) and both at a relative humidity of 70 to 80 percent. The third group was stored at a mean temperature of 22.8 C (range, 21 to 24 degrees) in a desiccator provided with a saturated solution of ammonium sulphate to give a relative humidity of 74 to 80 percent. Fed and unfed adults and nymphs were stored in 50 ml flasks provided with a layer of fine dirt while the larvae were stored in glass shell vials. These containers were stoppered with plastic plugs or corks which were center-drilled to provide for air circulation. Strips of voile gauze were fixed on the bottoms of these stoppers to prevent the escape of the unfed larvae.

Rabbits were also used in feeding all stages of this species. A rabbit was prepared for use as in the feeding method described for *O. megnini.* The animal was then restrained within a V-shaped wooden rack (plate I, fig. 3). The V-rack (about 80 degree angle) had sides 2 feet long by 7 inches wide. Leg boards fitted into slits along the top edges of the rack to keep the rabbit's fore and hind legs extended. The nymphs or adults were placed on the shaved portion of the abdomen and covered with a petri dish of a size suitable to that of the rabbit and to the number of ticks used. The petri dish was held in place by a leather strap placed around the frame of the rack and over the top of the petri dish (plate I, fig. 4). Nearly all specimens completed engorgement within 1 hour after attachment. In a few instances second and third stage nymphs required removal by force. Although these specimens were fully engorged they remained at their attachment sites. The technique described by Kohls (1937) for feeding *D. andersoni* was used to feed larvae of *O. coriaceus.* A rabbit was anesthetized with Nembutal Sodium, the mid-region of the body closely shaved, and an adhesive tape girdle containing a metal cage (a screw top lid from a 5-gallon can) was firmly attached (plate II, fig. 2). The zipper girdle described for feeding larvae of *O. megnini* was also used with larvae of *O. coriaceus.* In this case a greater proportion of fed specimens was obtained. Since the majority of larvae completed engorgement by the seventh day after application, the girdle was removed and the remaining specimens were removed by force. These were discarded since they had not completed feeding and since observations showed that such ticks eventually died without molting. In a few instances, larvae were also fed on the forearm of a man by means of a one-cage adhesive girdle.

O. moubata: For rapid development of this species, all eggs were maintained in a 32 C cabinet. Adults and nymphs were stored at a mean temperature of 32.6 C at a relative humidity of 51 to 61 percent. Eggs were kept in small shell vials containing a layer of fine sterile sand. Nymphs and adults were stored in similar vials containing a layer of fine dirt. After the colony reached sufficient numbers, it was maintained at room temperatures (as with *O. coriaceus*) in a desiccator provided with a small amount of water at the bottom to keep the humidity between 50 and 61 percent. Since the larvae do not feed, the nymphs and adults were placed on the shaved abdomen of a rabbit as was done in feeding *O. coriaceus.* This procedure proved more efficient than previously used feeding methods on fowl (Cunliffe and Nuttall, 1921) or on the ears of

rabbits (Jobling, 1925).

A. americanum and D. andersoni: A technique described by Morton (1929) and later outlined by Kohls (1937) proved satisfactory for feeding these species. A similar technique was used by Sapre (1943) for feeding *Boophilus* and *Rhipicephalus* specimens on goats and dogs. The two-cage method was used in order to feed a large number of specimens per rabbit host. This method utilized an adhesive tape girdle containing two metal cages (screw top lids from a 5-gallon can) bound around the shaved abdomen of a rabbit (plate II, fig. 2). Rabbits used for feeding these species could be kept for only three to four feedings. The refusal by ticks to attach became pronounced on subsequent feeding trials. Similar results with the same species and host animal were obtained by Kohls (personal communication). Rabbits unsuitable for further feeding by the ixodids were then used for feeding specimens of argasids.

The larvae and nymphs of each species were fed as were the adults. A modified "tick picker" from one described by Kohls (1937) was made in order to facilitate feeding (plate II, fig. 4). A sheet metal funnel was soldered to the base rim of a garbage can the bottom of which had been cut out. Cast iron legs were placed around the base. Strips of cast iron were placed at right angles to each other and fixed just below the top, inside wall. A 0.5-inch gauge wire screen cage was placed on top of the iron supports. A 6-mesh, circular wire screen was placed on small angle iron supports which were located one foot below the top strips of iron used to hold the animal cage. A 14-mesh, circular wire screen was placed 4 inches below the 6-mesh screen. When in use, this so-called "Berlese funnel" was put on top of the tick-barrier table.

A rabbit was anesthetized with Nembutal Sodium and placed in a bag (made from pillow ticking) and the specimens brushed from the holding vial onto the ears, neck, and back of the animal. Preliminary shaving of the animal around the head, nape of neck, and along the back facilitated attachment. To prevent loss of specimens from the vial or contamination of the handler, pentachlorocyclohexane was applied to the outside rim of the vial, to the handle of the brush, to the drawstring area of the bag, and around the wrists of the handler. Removal of the highly mobile larvae from the vials was facilitated by placement of the vials, just prior to transfer of specimens to the host, on top of a refrigerator ice tray for 1 minute. Upon completion of the transfer the bag was closed and placed in a holding cage. Approximately 12 hours later the animal was removed from the bag and placed in the top of the Berlese funnel. A muslin bag was placed over the cage to prevent ticks, shaken from the animal, from falling outside the funnel. Food was placed at frequent intervals inside the cage. The engorged specimens dropped off the animal and down through the screens where they came to rest on a strip of gauze covering the top of a jar attached to the bottom of the funnel. This arrangement enabled recovery of the ticks, free from feces, urine and waste food material. After feeding, the males were discarded and the females were placed in individual glass shell vials, the ends of which were stoppered with plastic plugs described for use with *O. coriaceus*. The vials were laid horizontally on a screen placed within a metal box containing sand. The box was placed on the tick-barrier table at room temperatures. The sand was moistened daily to provide a relative humidity of 50 to 61 percent. Fed larvae and nymphs were also stored in shell vials and kept at a mean temperature of 25.7 C (range, 23 to 26.5) and a relative humidity of 45 to 50 percent. This method accelerated development to the nymphal and adult stages. Nymphal molts occurred in 3 to 4 weeks under these conditions. To retard development, unfed adults, engorged females, unfed nymphs, and larvae were kept in either pill boxes or shell vials at 5 C. Since Gregson (1942) recommended a humidity of 70 to 75 percent, the containers were placed over a moistened box of sand as in the manner described for adults kept at room temperatures.

RESULTS AND DISCUSSION

Complete life cycles of ticks investigated are shown in table I. Almost 70 percent of *A. persicus* developed from egg to adult in 7 weeks when the colony was kept at 25 C. Herms (1950) states that adults can be obtained in 30 days if the colony is maintained at 21 C. Duplication of such a short cycle was not obtained by the present writer. At room temperatures the shortest cycle was 8 weeks. These results were similar to those reported by Micks (1951) from colonies kept at temperatures ranging from 25 to 28 C and humidity between 70 and 80 percent. The method of feeding ticks used by Micks, however, does not allow obtaining specimens of known age. In the present study a larger proportion of specimens attached and engorged on their hosts when given at least a 24-hour interval after each molt before feeding. Likewise, more fed specimens were obtained when the

TABLE I. *Developmental rates of argasids and ixodids under laboratory conditions: Number of days at centigrade temperatures.**

Stage of development	*Argas persicus* 32 degrees	*Otobius megnini* 21–24 degrees	*Ornithodoros coriaceus* 32 degrees	*Ornithodoros coriaceus* 25 degrees	*Ornithodoros coriaceus* 21–24 degrees	*Ornithodoros moubata* 32 degrees	*Dermacentor andersoni* 21–26 degrees	*Amblyomma americanum* 21–26 degrees
Period to oviposition	6–10	6– 12	15	35	55	8– 14	8– 22	7– 16
Egg incubation	2– 4	14– 19	23	32	40	7– 10	24– 28	24– 31
Interval**	3	14– 21	3	3	7	††	14– 21	7– 14
Larval feeding	5– 9	***	7 to 10 days			††	3– 6	3– 7
Larval molt	7–10	9– 13	10	16	28	3– 6	16– 27	9– 27
Interval**	1	none	none			2	7– 10	4– 6
Nymphal feeding	—	—	—	—	—	—	6– 8	3– 6
First nymph molt	7–10	7– 12	8– 12†	10– 19	16– 39	8– 17	18– 34	14– 19
Interval**	1	***	none			none	7– 9	3– 6
Second nymph molt	7–10	12– 41	14– 19	18– 26	21– 30	9– 20		
Third nymph molt	none	none	10– 14	16– 21	24– 60	10– 19		
Fourth nymph molt	"	"	16– 45	22– 56	23– 74	7– 30		
Fifth nymph molt	"	"	28– 49	28– 55	25– 92	10– 15		
Sixth nymph molt	"	"	35– 62	39– 86	65–114	12– 22		
Seventh nymph molt	"	"	33– 52	none	53 (one)	none		
Adult feeding	—	—	—	—	—	—	7– 12	10– 16
Total days for complete cycle:	39–58	62–118	202–314	226–359	364–602	76–155	110–177	84–148

* See text for range and for relative humidities.
** To allow for cuticle hardening.
*** On host.
† First nymphal molts to second without feeding.
†† Larva does not feed.

larvae were given at least a 72-hour interval between egg hatching and feeding.

For *O. megnini* kept at room temperatures oviposition occurred with most females between 6 and 12 days after dropping as nymphs from their hosts. Although Herms (1917) reported that it took 18 to 23 days for eggs to hatch, a shorter interval was obtained in the present study. From the feeding trials on bovines by Hooker (1908), the molting of larvae was recorded at 7 days while the majority of larvae molted 9 and 10 days after attachment. Present larval records agree with those by Hooker. Hooker further records the majority of nymphs dropping in 3 to 4 months. The present writer obtained nymphs between 2 and 4 months after attachment, but this period includes a 2- to 3-week interval between egg hatching and feeding for hardening and development of the cuticle. The occurrence of two nymphal cast skins from specimens in the present study supports the latter evidence that this species undergoes two nymphal stages (Cooley and Kohls, 1944). Bacha (1957) recorded only one nymphal stage for *Otobius lagophilus* Cooley and Kohls, the only other species in this genus. Bacha's colony was kept at room temperature (median, 16 C. humidity of 81 percent) and the life cycle varied between 55 and 134 days. This cycle is comparable to that for *O. megnini* found in the present study and suggests an unusually long first nymphal period if not two nymphal stages for *O. lagophilus*.

The results with *O. coriaceus* illustrate the wide variation in development. Acceleration of the life cycle was obtained at temperatures higher than those in the laboratory due to a decrease in the period of time normally experienced at room temperatures for the first through fourth nymphal stages. In most cases specimens kept at room temperatures molted to adults after the fifth nymphal stage. After 1 year, ticks kept at 32 and 25 C were transferred to variable room temperatures and maintained as was the third group. Pregnant females and eggs were returned to the 32-degree cabinet when acceleration of oviposition and egg incubation were required. The colony of Herms (1916) kept at 26 C produced from three to six

nymphal stages in a life cycle of 15 months. Nuttall (1911) and Smith (1944) recorded seven nymphal stages, the majority of adults occurring after the fifth and sixth nymphal molts. The results from the present study agree with those of Nuttall and Smith, and are similar to those by Davis (1953) for *O. braziliensis* Aragão. Smith (personal communication), however, maintained colonies of *O. coriaceus* at various room temperatures and in constant temperature cabinets (with uncontrolled relative humidity); the ticks developed at a uniform rate at any one given temperature. With the exception to the increased rate of oviposition at high temperatures as mentioned above, similar results to those by Smith occurred with specimens used in the present study. Larvae fed on the forearm of the present writer but only after many trials. The larvae that fed died without molting.

The life history and biology of *O. moubata* is well known (Cunliffe and Nuttall, 1921; Davis, 1947), but the number of nymphal stages has been found to vary between six (Newstead et al, 1907) and nine (Herms, 1950). Six nymphal stages occurred with specimens used in the present study.

Some workers have recommended that colonies be kept at 20 to 25.5 C and a humidity of 80 percent (Brett, 1939) or at a constant temperature of 30 C with a relative humidity of 50 percent or less (Jobling, 1925) or one as high as 80 percent (Robinson, 1942). In the present study the minimum time from egg to adult varied from 9 to 11 weeks with the colony kept at 32 C. At room temperatures, however, this period usually required 18 weeks.

A. americanum and *D. andersoni* are both three-host ticks. Their life cycle in nature usually requires 1 to 2 years, the ticks at each instar feeding a single time for 1 to 2 weeks while attached on different types and sizes of hosts. Under laboratory conditions this cycle was reduced to a minimum of less than 2.5 months (71 days) for *A. americanum*. Granett and Sacktor (1947) obtained a life cycle of 3 months for this species kept at 30 C and between 85 to 100 percent relative humidity. Lancaster (1955) recommended a relative humidity of 85 percent for adults kept at room temperatures. In the present study such high humidity was not found necessary. Lancaster (op. cit.) obtained a life cycle of 4 months (121 days) but felt that a minimum of 94 days was possible. In the present study, a minimum life cycle of slightly more than 3 months (96 days) was obtained for *D. andersoni*. Attempts were made to shorten the oviposition period of both species at 30 and 32 C. Gravid females of these species at room temperatures, however, oviposited in less time than the 25-day minimum recorded for *A. americanum* by Granett and Sacktor (op. cit.) and Lancaster (op. cit.). The feeding of larvae after a 1- to 3-week interval post egg-hatching allowed almost 100 percent of the larvae to complete cuticle hardening. In this manner, a large proportion attached to their initial host and larval mortality was reduced to a minimum. During the cuticle hardening period readiness to feed was indicated by the presence of white excretory material deposited by the larvae on the walls of the vials (Kohls, 1937). The storage of specimens in various stages of development in a cold room (5 C) provided a stock of ticks for future use and a delay in oviposition by fed females for as long as 4 months. Similarly, unfed adults were kept as long as 4 months, while unfed and fed nymphs, and larvae, were kept up to 2 months.

The colony techniques for each species used in the present study provided specimens of known age at any stage of development. Such techniques may be applicable to ticks reared for special studies (i.e. toxicological, physiological, etc.).

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SUMMARY

Colony rearing techniques are described for the ixodid species *Amblyomma americanum* (Linnaeus) and *Dermacentor andersoni* Stiles and the argasid species *Ornithodoros coriaceus* Koch, *Ornithodoros moubata* (Murray), *Argas persicus* (Oken), and *Otobius megnini* (Dugès). The 2-year life cycle of ixodid species was reduced to a minimum of 2.5 to 3 months, respectively, under laboratory conditions.

A successful and simple rearing technique for the spinose ear tick, *O. megnini*, on the closely shaved abdomen of rabbits is described. A cloth-zipper girdle was designed for feeding this species as well as for the larvae of *Ornithodoros* species which require several days feeding on a host. Two nymphal stages were found for *O. megnini*.

At 32 C minimum life cycles of 2.5 and 8 months were obtained for *O. moubata* and *O. coriaceus*, respectively. Results similar to those reported by earlier workers showed that the maximum number of nymphal stages was six for *O. moubata* and seven for *O. coriaceus*. Larvae of *O. coriaceus* died without molting after feeding on human blood.

A convenient technique for feeding larvae of *A. persicus* on birds is described. The life cycle of this species was between 7 and 8 weeks under laboratory conditions.

Prior to feeding the ticks on rabbits the animals were more easily handled when given a subcutaneous inoculation of Nembutal Sodium. The interval provided for larvae and nymphs to complete cuticle hardening permitted a larger proportion of specimens to engorge on a host.

The colony techniques provided specimens of known age at any stage of development.

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EXPLANATION OF PLATES

PLATE I

FIGURE 1. Tick-barrier table with colonies at room temperatures.
FIGURE 2. Restrained chicken with *A. persicus* applied and ready for transfer to crock.
FIGURE 3. V-rack animal-holding board; a, belt, b, petri dish, c, rack, d, leg-boards.
FIGURE 4. *Ornithodoros* species on rabbit in V-rack.

PLATE II

FIGURE 1. Rabbit on holding board with zipper-cloth girdle for feeding larvae and nymphs of *O. megnini* and larvae of *O. coriaceus*.

FIGURE 2. Adhesive tape girdle with metal screw-top lids for feeding adults and nymphs of *O. coriaceus* and *O. moubata* and adults of *A. americanum* and *D. andersoni*.

FIGURE 3. Chicken cage used when feeding larvae of *A. persicus:* a, wash pan; b, 5-gallon metal container; c, food and water trays; d, metal screen; e, black muslin cover.

FIGURE 4. Modified Berlese funnel with rabbit in small holding cage for feeding immature ixodid species.

PLATE I

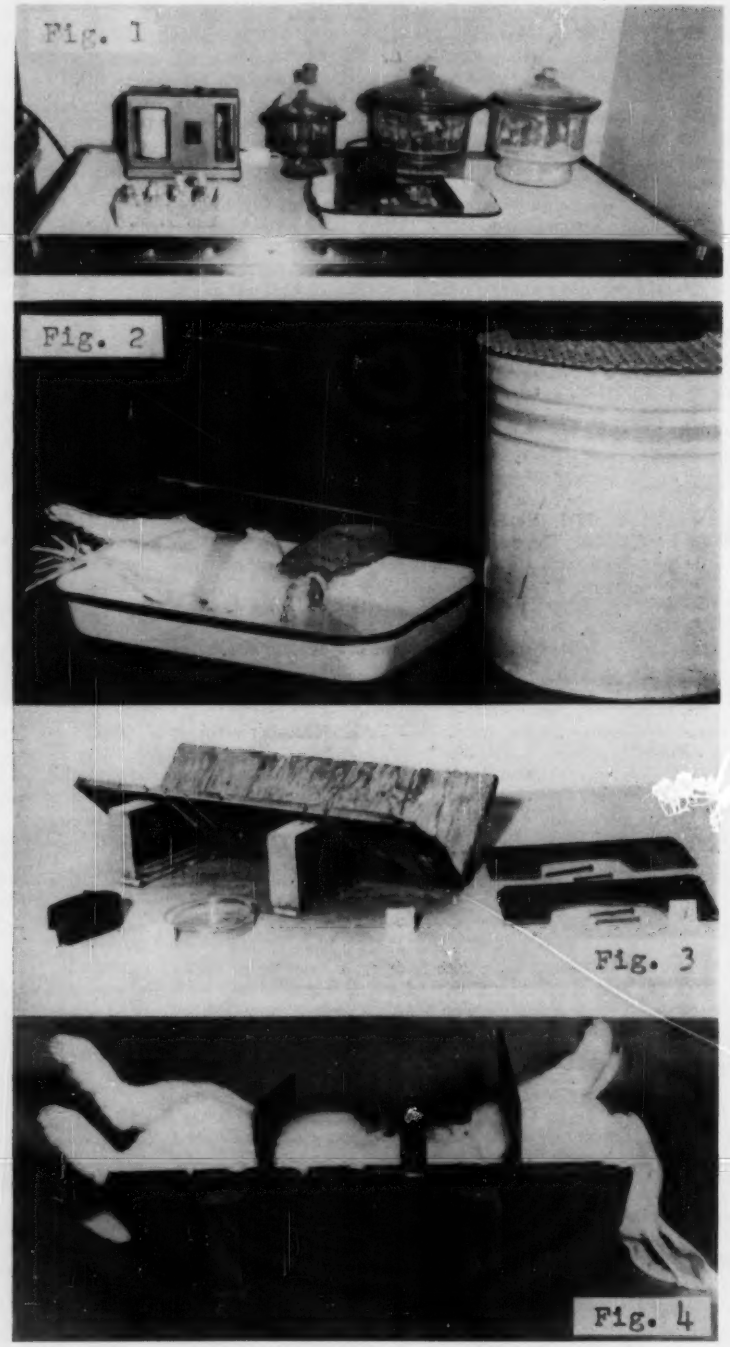
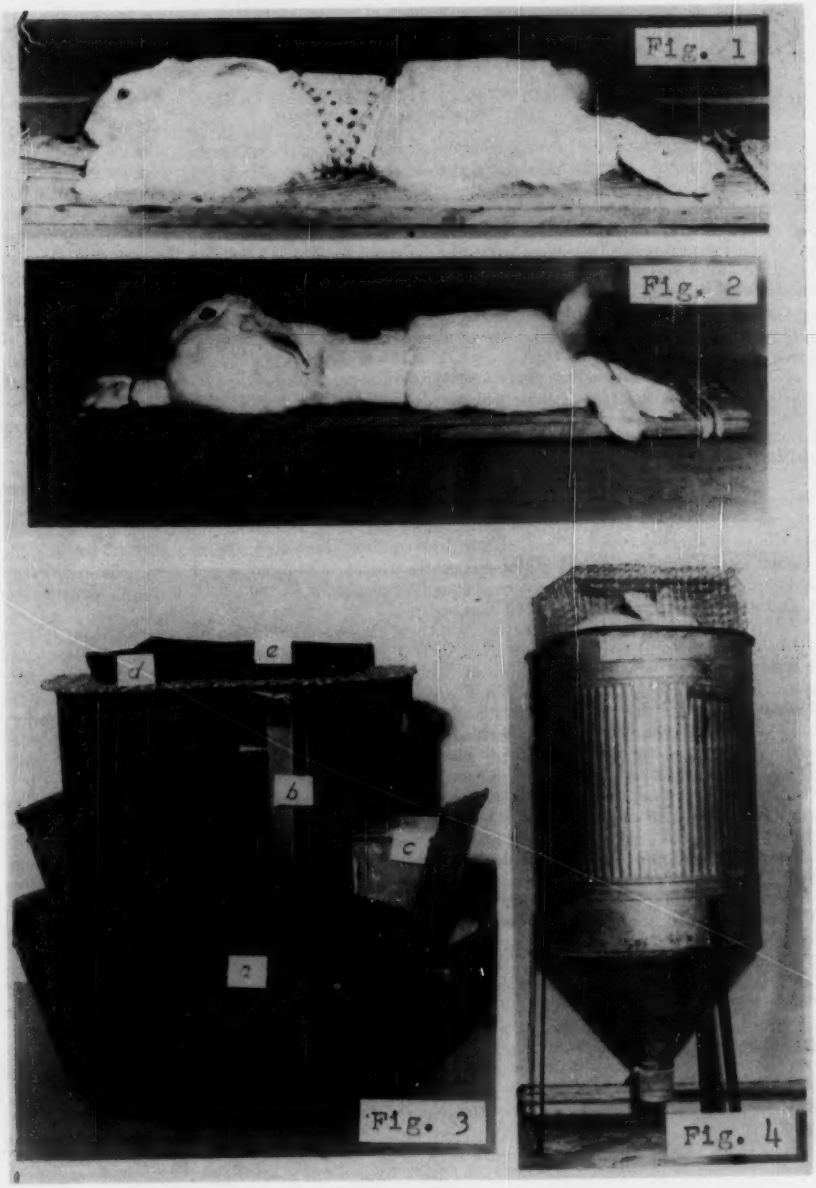


PLATE II



RESEARCH NOTE

OBTAINING FREE-ENCYSTING METACERCARIAE (TREMATODA: DIGENEA) FOR TECHNICAL STUDY.

A preliminary survey of cyst formation among digenetic trematodes (Kruidenier, 1953, J. Parasit. **39** (supp.): 16) included examples of those cysts which are formed upon inanimate objects in the environment of the infected snail hosts. The generally suggested technique of allowing infected snails to shed the cercariae into cups of lettuce upon which the cercariae could encyst was used at first to obtain necessary specimens. Metacercariae were subsequently studied in situ on the lettuce, or they were carefully dissected from this substrate for technical manipulation. Neither was satisfactory. It was always necessary to subdivide the lettuce into numerous small pieces for further histological treatment because of the dispersion of the specimens and the intractability of the lettuce. The in situ specimens were usually obscure. Even careful removal of the cysts destroyed portions of their generally fragile outermost walls. It was also difficult to observe encystation closely enough to be certain that the desired stages were obtained.

During subsequent studies (unreported), simple techniques were developed for obtaining undisturbed specimens which could then be concentrated and handled as desired without interfering substrates. These also provided specimens at predetermined, desired stages in cyst formation by permitting close observation of specimens and fixation at will.

In one technique, a glass slide was warmed and, while cooling, coated on one surface with a thin layer of embedding paraffin. Cercariae, placed on the paraffin surface, could be observed during encystation under dissecting microscopes or, with some trouble, under the compound microscope. Thus desired stages could be obtained. The specimens fixed under these conditions were satisfactory for further histological studies. However, the friability of the paraffin and its poor attachment to the slides frequently caused it to become detached. Desired specimens thus floated to the surfaces of reagents and ready exchange of test and dehydrating solutions was interfered with.

A parlodion film was subsequently substituted for the paraffin coating. Slides were placed briefly in 0.01 percent paraloidin dissolved in 1:1 ether-alcohol solvents from an absolute ethyl alcohol rinse. Air dried to initial firmness while draining in a vertical position, they were then placed in 85 percent alcohol to harden finally, and stored back to back in coplin jars of the alcohol or of distilled water. All slides were rinsed thoroughly in running tap water before use. Where large numbers of fully formed cysts were desired, the snail containers were coated on the inside, as above, by rinsing them with the parlodion solution. They were not allowed to air dry completely before use as the film tends to detach under these conditions.

Specimens could be studied readily with the compound microscope (high-dry lenses) during encystation. Careful adjustment of the water on the cercariae and the use of similarly small amounts of fixative made it possible to observe closely and to use the same slides for several individuals or groups. Even relatively large drops of fluids tend to retain their identity on the films. The specimens were subsequently handled like slides of sectioned tissues through all but the final dehydration in absolute alcohol in which dissolution of the film detaches the specimens. Where the latter was not desired, several drops of chloroform in the alcohol preserved film and the attachment of the metacercariae. Chloroform, of course, is contraindicated in some histochemical procedures.

The transparency and durability of the parlodion films made them more generally useful. Either technique was preferable to the use of lettuce as a substrate. (This investigation was supported in part by a research grant (E-1319) from the National Institute of Health, Public Health Service.)—F. J. KRUIDENIER, *Zoology Department, University of Illinois, Urbana.*

STERNOSTOMA SIALIPHILUS N. SP. (ACARINA: RHINONYSSIDAE) FROM THE NASAL CAVITIES OF THE EASTERN BLUEBIRD, *SIALIA SIALIS* (LINNAEUS)* **

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Four specimens of the Eastern bluebird, *Sialia sialis* (Linnaeus), family Turdidae, from the vicinity of the Kellogg Gull Lake Biological Station, Kalamazoo County, Michigan, were examined for nasal mites. One bird had two female mites, another was infested with three female mites. These mites represent a new species of *Sternostoma* Berlese and Trouessart, 1889, and are described herein. With this description the number of species in the genus *Sternostoma* known to occur on the North American continent is increased to seven. Of these, *Sternostoma spatulatum* Furman, 1957, from the olive-backed thrush (*Hylocichla ustulata* (Tschudi)) is the only other species of this genus reported from birds of the family Turdidae.

Variation in paratypes is given in parentheses. The measurements recorded are in microns.

Sternostoma sialiphilus n. sp.

Female (Figs. 1 to 5)

Dorsum (fig. 5): Idiosoma of holotype 490 (390 to 590) in length, 253 (280 to 300) in width. Podosomal plate 207 (198) in length, 197 (184 to 190) in width. Podosomal plate rounded anteriorly, with slightly convex lateral borders and posterior margin nearly straight. Plate granular with vacuolate areas and with nine pairs of alveoli, each bearing a microseta as figured (fig. 5).

Opisthosomal plate quadrangular, slightly wider than long, narrowing posteriorly; 130 (123 to 125) in length, 140 (137 to 140) in width. Plate granular, vacuolate, with four pairs of alveoli, the posterior pair each bearing a microseta.

Cuticle finely striated with one pair of minute posterior, submarginal setae and three pairs of indistinct contiguous alveoli situated between podosomal and opisthosomal plates. Stigmata located near the posterior corners of the podosomal plate, circular (10 in diameter), and without peritremes.

Venter (fig. 1): Sternal plate rectangular with poorly defined margins; 128 (117 to 121) in length, 68 (65 to 70) in width. Surface punctate with three pairs of alveoli, each alveolus bearing a microseta. Cuticle finely striated with two pairs of minute setae.

Genito-ventral plate relatively broad, with almost straight lateral margins, broadly rounded posteriorly, with vacuolate pattern in mid-region and without genital setae; 95 (92 to 96) in in length, 62 (56 to 70) in width.

Anal plate bearing one pair of stout adanal setae; cribrum posterior to anal opening. Plate readily measurable only on one paratype, being 55 in both length and width.

Gnathosoma (fig. 2): Ventral in position with distal half visible in dorsal view; 97 (93 to 96) in length (including palps). Palps 4-segmented, the distal segment bearing three large

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blunt setae and approximately five smaller setae. Basal, second, and third segments bearing 0, 3, and 4 setae respectively. Length of palps from basal segment to tip of tarsus 56 (51 to 60). Chelicerae 54 (54 to 55) in length, 10 in width, broad basally, attenuate; chelae minute, approximately 3 in length. Setae lacking on remainder of gnathosoma.

Legs: All legs 6-segmented, moderately sclerotized, leg I being somewhat stouter than others. All tarsi with caruncle and claws, claw I being highly modified. Tarsi II, III, and IV having a well-defined pseudoarticulation. Leg measurements, from base of coxae to tip of caruncles, as follows: leg I—365 (341 to 367), leg II—254 (232 to 261), leg III—281 (264 to 285), leg IV—336 (321 to 334). Approximate length of claws, 20.

TABLE I. *Chaetotaxy of legs.*

Leg No.	Coxa	Troch.	Femur	Genu	Tibia	Tarsus
I	2(2)	3(3)	6(6–7)	6(6–7)	6(6–7)	23(20–23)
II	1(2)	3(3)	5(5–7)	5(4–6)	6(6)	14(14–15)
III	1(2)	3(3)	4(3–5)	4(4–6)	6(5–7)	14(14–16)
IV	1(1)	3(3)	4(4)	5(4–5)	7(5–7)	14(11–18)

Exclusive of the tarsal setae, the leg setae are short (1 to 3) and blunt. Tarsus I (fig. 4) with 4 attenuate setae, 1 long sensory rod, 1 heavy spur-like subapical seta, and approximately 17 smaller setae. Tarsus II (fig. 3), III, and IV each with two attenuate setae, five spatulate setae, and approximately seven short, less differentiated setae.

Diagnosis: Sternostoma sialiphilus appears to be most closely related to *S. hirundinis* Fain, 1956 (Hosts: *Psalidoprocne albiceps* and *Hirundo smithi*, Ruanda-Urundi). It can be separated from *S. hirundinis* on the basis of the following characters: (1) *S. sialiphilus* lacks the pair of ventral gnathosomal setae present in *S. hirundinis*, (2) the vacuolate pattern of the genito-ventral plates differs, and (3) the sclerotization and setal number of the anal plate differs. (See also Fain, 1956.)

Fain figured two setal types on tarsi II, III, and IV in his description of *S. hirundinis*. In *S. sialiphilus* these tarsal setae resemble more closely those from the host *Hirundo smithi*.

In addition, *Sternostoma cryptorhynchum* Berlese and Trouessart, 1889, known in Europe from the English sparrow, *Passer domesticus*, is considered closely related to *S. sialiphilus*. It differs from *S. sialiphilus* in the number of dorsal opisthosomal setae, the lack of a vacuolate pattern on the genito-ventral plate, and the presence of a sclerotized pattern on the ventral aspect of the gnathosomal base. In *S. cryptorhynchum* the opisthosomal plate is longer than wide. (See also Berlese and Trouessart, 1889.)

MATERIAL

Type Host: Eastern bluebird, *Sialia sialis* (Linnaeus).

Type Locality: Kalamazoo County, Michigan, U. S. A.

Type Specimens: Three female specimens were taken from a juvenile bluebird collected at Brook Lodge Road and County Road 400, Kalamazoo County, Michigan on 8 July 1959. One of these has been designated the holotype; one, a paratype; the third was inadvertently destroyed. Two females were recovered from a bluebird collected at "B" Avenue and Kalamazoo County Line Road, Kalamazoo County, Michigan on 21 July 1959. Collections were made by David T. Clark, K. E. Hyland, and H. G. Ford.

Disposition of Material: The holotype is deposited in the United States National Museum (U.S.N.M. No. 2659); paratypes are deposited with Alex Fain, Institut de Médecine Tropicale, Antwerp, Belgium, and in the collections of the authors.

SUMMARY

A new rhinonyssid mite, *Sternostoma sialiphilus*, is described from female specimens taken from the nasal cavity of the Eastern bluebird, *Sialia sialis*, collected in Kalamazoo County, Michigan.

LITERATURE CITED

BERLESE, A. AND TROUESSART, E. L. 1889 Diagnosis d'acariens noveaux ou peu connus. Bull. Soc. Sci. Nat. Ouest France (Nantes) **2**: 126–130.

FAIN, A. 1956 Les acariens de la famille Rhinonyssidae Vitzthum, 1935, parasites des fosses

nasales des oiseaux au Ruanda-Urundi (note preliminaire) Rev. Zool. Bot. Afr. **53**: 131–157.

EXPLANATION OF PLATE I

Sternostoma sialiphilus n. sp.

FIGURE 1. Female, ventral view.
FIGURE 2. Female gnathosoma: Right, dorsal view; left, ventral view.
FIGURE 3. Tarsus II of female, ventro-lateral view.
FIGURE 4. Tarsus I of female, dorso-lateral view.
FIGURE 5. Female, dorsal view.

RESEARCH NOTE

TWO CASES OF HUMAN ASCARIASIS IN NEW HAMPSHIRE.

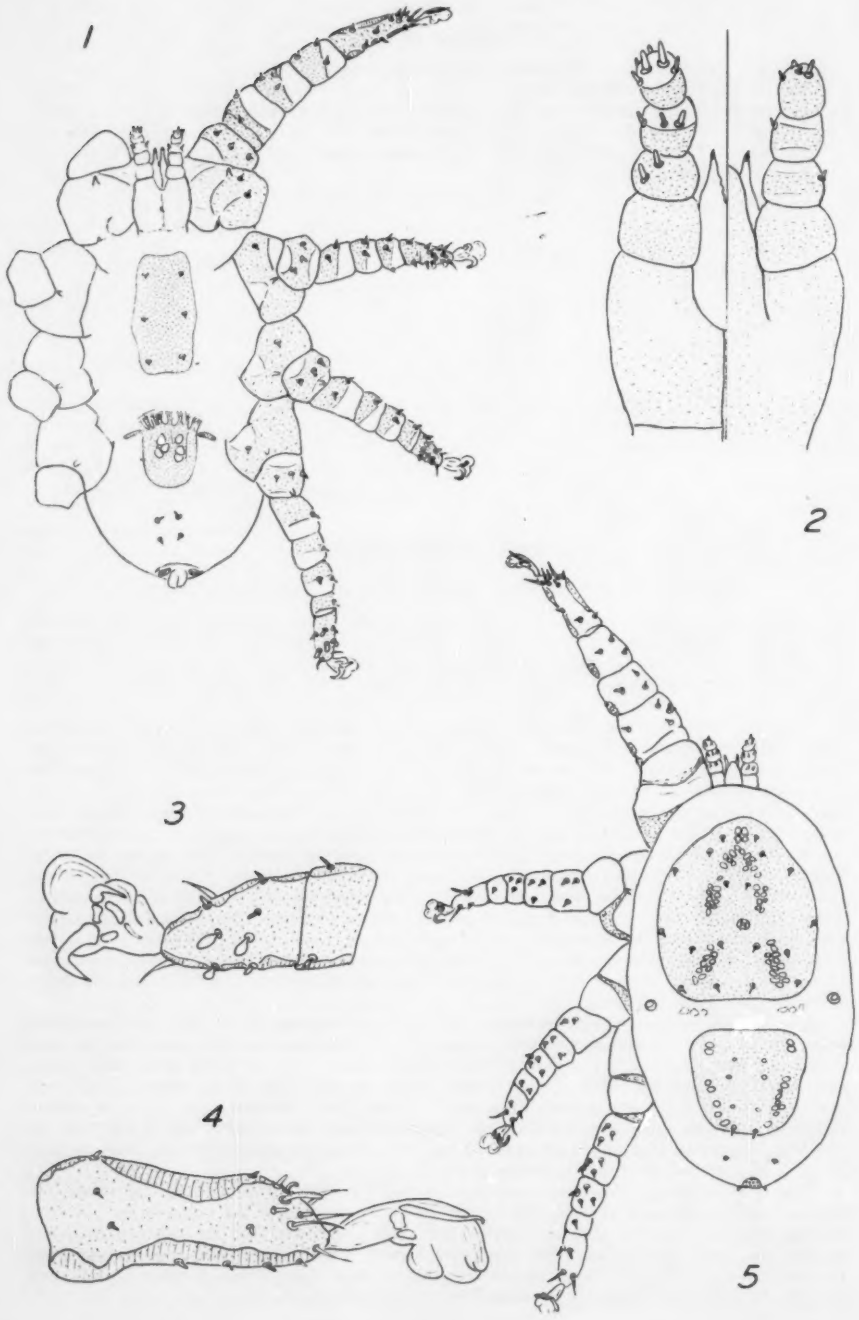
Two cases of human infection with *Ascaris lumbricoides* have been observed in New Hampshire in recent years. Since most references do not indicate that New England is in the geographic range of true human ascariasis, and since most evidence reported thus far implies that pig ascaris develops only rarely in man, these cases were followed up over a 2- to 3-year period.

Case No. 1, a girl from Laconia, N. H., became infected with Ascaris for 3 successive years beginning prior to her second birthday. Each year the infection was manifested by rather severe attacks of asthma. Elimination of one or two large ascarids completely alleviated the symptoms each time. No other cases of ascariasis had been reported from the family or the surrounding area, nor were pigs anywhere on the premises. Examination of soil samples from several parts of the property yielded a considerable number of mature, embryonated ascaris ova in the soil of the garage floor and the driveway leading from it. The garage had been used as a pig pen by previous tenants 5 years prior to the first infection. Since the girl did not play in the garage, she appears to have picked up the infection while playing in the driveway. Although the first two infections failed to produce sexually mature worms, the third infection resulted in the production of normal ascaris eggs that were observed in the stool prior to the removal of several mature worms. While the final outcome of this case is not known, the parents had planned to pave both the garage floor and the driveway. No further complaints of ascaris have been reported.

Case No. 2 is that of an 18-month-old girl from Nottingham, N. H. This child exhibited more typical ascaris symptoms: Anemia, stomach pains, etc. Eggs had been found in the stool by a local physician and a few mature worms were subsequently removed. Since this family had been in the town a number of years and there was no knowledge of pig raising in the area, soil samples were examined. Ascaris eggs were recovered from the sand (brought in to make a sand box) in which the child played. A few eggs were found in the native soil uphill from the sandbox. No further study has been made of this case and, apparently, there has been no more trouble; but the origin of this infection is not at all clear.

While in neither of these two cases is it certain that the infection is of pig origin, the circumstantial evidence is considerable in case No. 1. In case No. 2 the evidence for involving the pig is quite weak. In addition, there remains the possibility that the soil contamination in this case may have come from the child herself. The problem still remains unsolved in that the child had never been outside the state of New Hampshire.—WILBUR L. BULLOCK, *Zoology Department, University of New Hampshire, Durham, N. H.*

PLATE I



NEW GENERA AND SPECIES OF CHIGGERS FROM PANAMA (ACARINA: TROMBICULIDAE)

JAMES M. BRENNAN AND ELEANOR K. JONES

U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana

Among numerous lots of chiggers received for identification from Maj. Gordon Field, Capt. Vernon J. Tipton, and Dr. Carl B. Koford were 3 new genera and 17 new species which are here described and figured. Most distinctive is *Polylopadium* new genus, unique among chigger genera in that the larva has, in addition to the scutum, 13 punctate dorsal plates devoid of setae.

Holotypes are deposited in the Rocky Mountain Laboratory (RML); available paratypes there, in the United States National Museum (USNM), Chicago Natural History Museum (CNHM), British Museum (BM), and South Australian Museum (SAM).

The many other new records obtained from this material bring the number of recognizable Panamanian forms to nearly 50. These will be treated in a later paper.

All recorded measurements are in microns.

Odontacarus fieldi new species

(Figure 1)

Type data: Holotype and a paratype, RML No. 35284, off *Zygodontomys cherriei*, Fort Kobbe, Canal Zone, 8 June 1954; 1 paratype, off *Sigmodon hispidus*, Fort Clayton, C. Z.; 24 June 1954; Gordon Field, collector.

Holotype, RML; paratypes RML and USNM.

Diagnosis: A mastitarsala III, 2 genualae I, no parasubterminala, no genualae II or III, a microgenuala II, sensillae barbed, about 40 dorsal setae. Differs from *O. cayolargoensis* Brennan by nude galeal seta, a microgenuala II and III.

Body: Broad-ellipsoidal. Eyes 2/2, in a plate, the anterior larger. Length and width of holotype, engorged, 515 by 385. Anus at third row of ventral setae. Spiracles prominent.

Gnathosoma: Puncta few and scattered. Blades with dorsal and ventral rows of teeth as figured. Palpal setae B/B/BNN; tarsus with 7 branched setae and a tarsala; claw trifurcate. Galeal seta nude.

Scutum: As figured, with few puncta and sparsely barbed sensillae. Measurements of holotype: AW, 65; PW, 88; SB, 25; ASB, 28; PSB, 16; AP, 20; AM, 42; AL, 44; PL, 64; S, 66.

Legs: Sparsely punctate. Specialized setae as figured, a microgenuala II and no parasubterminala. Nonspecialized setae moderately to densely branched.

Body setae: Dorsal setae about 40, similar to scutals, 42 to 60, increasing in length laterally and posteriorly. Ventral setae 2 sternals plus about 38, postanals similar to dorsals.

Named for the collector, Maj. Gordon Field, United States Army.

Aniatrus new genus

Trombiculine larvae superficially like *Schöngastia* and *Schoutedenichia*. Cheliceral blade with a dorsal row of large teeth. Palpal tarsus with 6 branched setae and a tarsala. Scutum trapezoidal and with expanded sensillae. Eyes present. Leg segmentation 7-7-7. No genualae II or III, a tibiala III, no mastitarsala III.

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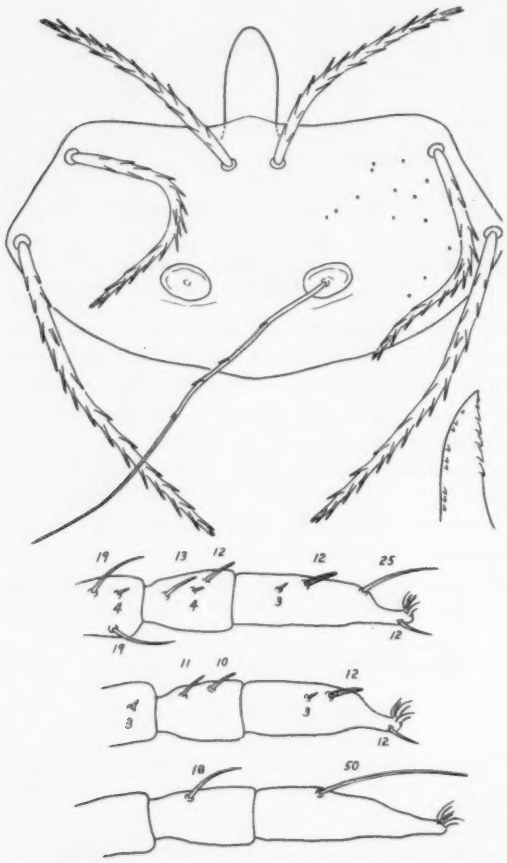

FIGURE 1. *Odontacarus fieldi* n. sp. Scutum, cheliceral blade, specialized setae of legs with measurements in microns.

Aniatrus bifax new species

(Figure 2)

Type data: Holotype, RML No. 35904, off armadillo (*Dasypus novemcinctus*). Albrook Air Force Base, Canal Zone, 17 March 1959, Vernon J. Tipton, collector. In the Rocky Mountain Laboratory.

Diagnosis: Two genualae I, no genualae II or III, no mastitarsala III, sensillae obovate, widely separated, ventral setae extend to first pair of sternals.

Body: Ovate. Eyes small, 2/2 in a plate. Length and width of holotype, apparently unengorged, 250 by 165. Anus at about sixth row of ventral setae.

Gnathosoma: Usual structures punctate. Cheliceral blades with dorsal row of 9 large teeth. Palpal setae B/B/BBB; claw trifurcate; tarsus with 6 branched setae and a tarsala. Galeal seta long, nude.

Scutum: As figured, anteromedian seta far behind margin, sensillary bases widely separated. Measurements of holotype: AW, 64; PW, 91; SB, 55; ASB, 22; PSB, 20; AP, 29; AM, 34; AL, 26; PL, 33; S, 31.

Legs: Punctate. Specialized setae as figured. Nonspecialized setae sparsely branched.

Body setae: Anterodorsal setae similar to posterolateral scutals, posterodorsals similar to anteromedian scutal seta, 27 to 34, increasing in length posteriorly, 2 humerals plus about 56.

Ventral setae 2-2 sternals plus about 60, postanals similar to posterodorsals. Ventrals extend as far as first pair of sternals.

A. bifax differs from species of *Schöngastia* in lacking a mastitarsala III and by the more shallow scutum, from species of *Schoutedenichia* in having a tibiala III and lacking genualae II and III. Species of the latter genus are usually intranasal parasites.

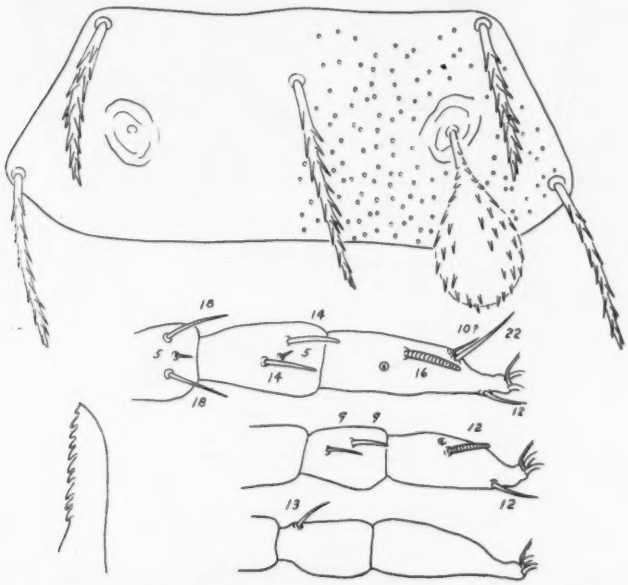

FIGURE 2. *Aniatrus bifax* n. sp. Scutum, specialized leg setae, cheliceral blade.

Ascoschöngastia dyscrita new species

(Figure 3)

Type data: Holotype and 15 paratypes, RML No. 35230, off *Liomys adspersus,* National Forest, Canal Zone, 21 December 1954; 2 paratypes, same host, Curundu, C. Z., 8 July 1954; 1 paratype, same host, National Forest, 9 March 1955; 4 paratypes, off *Sigmodon hispidus,* National Forest, 20 April 1955; 2 paratypes, off *Oryzomys talamancae,* National Forest, 4 March 1955; 2 paratypes, off *O. talamancae,* Cerro Azul, Panama, 8 February 1956; 1 paratype, off *O. caliginosus,* Cerro Azul, 26 January 1956; Gordon Field, collector; 1 paratype off *Proechimys semispinosus,* Bocas del Toro, Panama, 27 January 1960, Vernon J. Tipton, collector. Holotype, RML; paratypes, RML, USNM, BM, CNHM, SAM.

Diagnosis: Sensillae obovate, palpal setae branched, 2 genualae I, dorsal formula begins 2–10.

Body: Ellipsoidal. Eyes 2/2, in a plate. Anus at fifth row of ventral setae. Length and width of holotype, nearly engorged, 412 by 268.

Gnathosoma: Punctate. Blades with tricuspid cap. Palpal setae B/B/BBB, sparsely branched; claw trifurcate; tarsus with 5 branched setae, a subterminala and a tarsala. Galeal seta nude.

Scutum: As figured. Measurements of holotype: AW, 50; SB, 26; ASB, 22; PSB, 18; AM, 24; AL, 29; PL, 36; S, 33.

Legs: Punctate. Segmentation 7–7–7. Specialized setae as figured. Nonspecialized setae sparsely to moderately branched.

Body setae: Dorsal setae similar to scutals, 24 to 31, increasing in length posteriorly, arranged approximately 2–10–10–10–8–8–2, with occasional odd setae between the rows. Ventral setae 2–2 sternals plus about 48, postanals similar to dorsals.

FIGURE 3. *Ascoschöngastia dyscrita* n. sp. Scutum, and specialized setae of legs.

This appears to be the first record of a species of *Ascoschöngastia* in the Western Hemisphere. *A. dyscrita* is assigned to the genus on the basis of extrascutal PL's, leg segmentation 7–7–7, and no ventral humeral setae.

Euschöngastia cunctata new species

(Figure 4)

Type data: Holotype and a paratype, RML No. 35338, off *Oryzomys talamancae*, Cerro Azul, Panama, 8 February 1956; 6 paratypes, same host and locality, 8 and 14 February 1956, Gordon Field, collector.

Holotype, RML; paratypes, RML, USNM, CNHM.

Diagnosis: A tibiala III, 2 genualae I, coxa III with 2 to 4 setae, ventral setae extend to first pair of sternals, scutum deep, sensillae capitate, PL's set close to AL's.

Body: Broad-ellipsoidal. Eyes 2/2, in a plate. Length and width of holotype, engorged, 555 by 412. Anus at about eighth row of ventral setae.

Gnathosoma: Punctate. Blades with tricuspid cap. Palpal setae B/B/BBB; claw trifurcate; tarsus with about 6 branched setae and a tarsala. Galeal seta nude.

Scutum: As figured, deep, AL's and PL's approximate, sensillae capitate, PL's fairly long. Measurements of holotype: AW, 52; PW, 58; SB, 15; ASB, 27; PSB, 19; AP, 11; AM, 24; AL, 47; PL, 64; S, 30.

Legs: Punctate. Specialized setae as figured. Nonspecialized setae moderately to densely branched. Coxa III with 2 to 4 setae.

Body setae: Dorsals about 100, humerals not distinct, slender with long branches, especially basally, fairly uniform length (33). Ventral setae about 100, extending forward and between coxae II and III so that sternals are not readily discerned, postanals not greatly different from preanals.

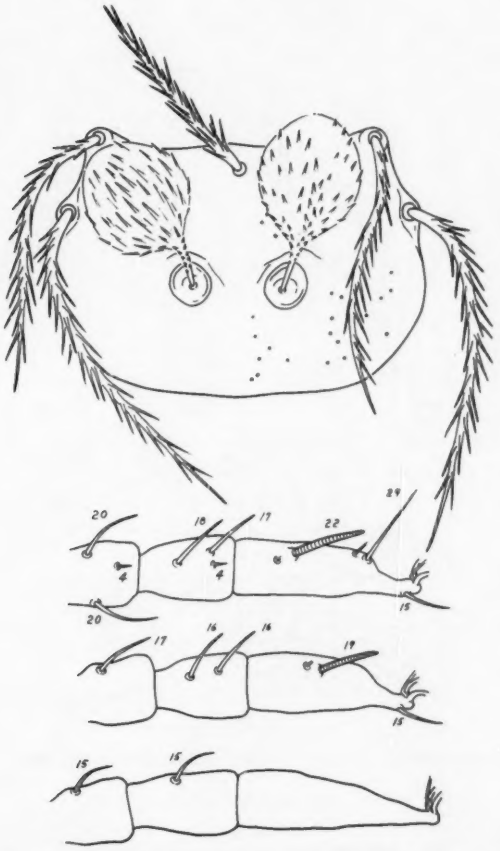

FIGURE 4. *Euschöngastia cunctata* n. sp. Scutum and specialized setae of legs.

Euschöngastia spissa new species

(Figure 5)

Type data: Holotype, RML No. 35963, off *Peromyscus nudipes,* Chiriqui, Panama, 4 May 1960. In the Rocky Mountain Laboratory.

Diagnosis: A tibiala III, 3 genualae I, scutum deep, with sinuous margins, sensillae narrow-ovate with long setules densely distributed, palpal genual and tibial setae nude.

Body: Ovate. Eyes large, 2/2, in a plate. Length and width of holotype, unengorged, 278 by 195. Anus at third row of ventral setae.

Gnathosoma: Punctate. Blades with minute subapical dorsal tooth and large ventral tooth. Galeal seta nude. Palpal setae B/N/NNN; claw trifurcate; tarsus with 7 branched setae, a subterminala and a tarsala.

Scutum: As figured. Sensillae appear shaggy because of densely distributed long setules. Measurements of holotype: AW, 74; PW, 90; SB, 41; ASB, 34; PSB, 18; AP, 23; AM, 45;

AL, 40; PL, 70; S, 42.

Legs: Punctate. Specialized setae as figured. Nonspecialized setae with long branches moderately to densely distributed.

Body setae: Dorsals thick, densely branched, like the scutals, 55 to 60 long, arranged 2-6-6-6-6-4-2. Ventral setae 2–2 sternals plus 18; sternals, preanals and postanals all of similar form.

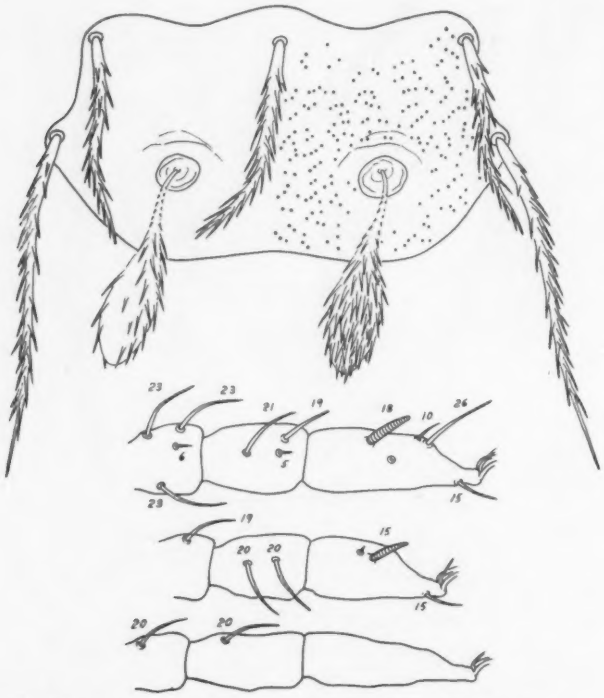

FIGURE 5. *Euschöngastia spissa* n. sp. Scutum and specialized leg setae.

Euschöngastia tragulata new species

(Figure 6)

Type data: Holotype and 4 paratypes, RML No. 34098, off *Nasua narica* (coati), Barro Colorado Island, Canal Zone, November 1956, Carl B. Koford, collector.

Holotype, RML; paratypes RML, USNM, CNHM.

Diagnosis: Scutum deep, sensillae lancelike, a mastitarsala III, 3 genualae I, dorsal formula begins 2–6.

Body: Ovate. Eyes 2/2, in a plate. Length and width of holotype, slightly engorged, 225 by 155. Anus at fifth row of ventral setae.

Gnathosoma: Punctate. Cheliceral blades with tricuspid cap. Galeal seta nude. Palpal setae B/B/NNB, dorso- and laterotibial occasionally forked; claw trifurcate; tarsus with 5 branched setae and a tarsala.

Scutum: As figured, deep, with narrow lanceolate sensillae. Measurements of holotype: AW, 50; PW, 60; SB, 21; ASB, 22; PSB, 24; AP, 26; AM, 34; AL, 23; PL, 33; S, 43.

Legs: Punctate. Specialized setae small, as figured. Nonspecialized setae sparsely branched.

Body setae: Dorsal setae slender, lightly branched, of fairly uniform length (28), arranged 2-6-6-6-6-4-2. Ventral setae 2–2 sternals plus 22, postanals similar to dorsals.

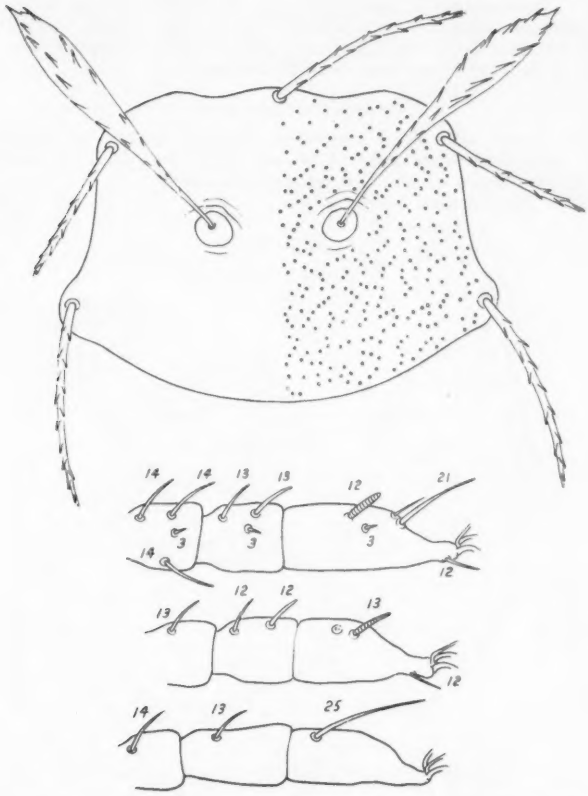

FIGURE 6. *Euschöngastia tragulata* n. sp. Scutum and specialized setae of legs.

Hoffmannina handleyi new species

(Figure 7)

Type data: Holotype and 5 paratypes, RML No. 35965, off *Peromyscus nudipes,* Department of Chiriqui, Panama, 4 May 1960; 84 paratypes off same host, *Peromyscus* sp., *Scotinomys teguina, Reithrodontomys mexicanus, Reithrodontomys* sp., and *Heteromys desmarestianus,* Chiriqui, 30 January to 4 May 1960; Vernon J. Tipton and C. Marvin Keenan, collectors.

Holotype, RML; paratypes RML, USNM, CNHM, BM, SAM.

Diagnosis: Seven scutal setae, sensillae flagelliform, a mastitarsala III, 2 genualae I. Separated from *H. suriana* (Hoffmann), the only other species of the genus, by 2 instead of 3 genualae I, and anterior PL's posterior instead of anterior to SB.

Body: Ovate. Eyes large, 2/2, in a plate. Anus at fourth row of ventral setae. Length and width of holotype 310 by 210.

Gnathosoma: Conspicuously punctate. Cheliceral blades with minute subapical dorsal and a larger ventral tooth. Galeal seta nude. Palpal setae B/B/NNB; claw trifurcate; tarsus with 7 branched setae, a subterminala and a tarsala.

Scutum: As figured. Measurements of holotype: AW, 77; PW, 95; PPW, 84; SB, 40; ASB, 30; PSB, 30; AP, 23; APP, 32; AM, 55; AL, 41; PL, 55; PPL, 50; S, 92.

Legs: Punctate. Specialized setae as figured. Nonspecialized setae densely branched to plumose.

Body setae: Dorsals about 50, similar to scutals, 36 to 53, decreasing in length posteriorly and increasing in length laterally. Ventral setae 2–2 sternals plus about 38, postanals like dorsals.

Named for Dr. Charles O. Handley, United States National Museum, who collected and identified many of the mammalian hosts listed in this paper.

Polylopadium new genus

Trombiculine larvae with dorsal pattern of punctate platelets in addition to scutum. Scutum with 5 small setae. Eyes absent. Cheliceral blades with tricuspid cap. Palpal tarsus with 5 branched setae and a tarsala. Leg segmentation 7–6–6.

Type species: Polylopadium kramisi n. sp.

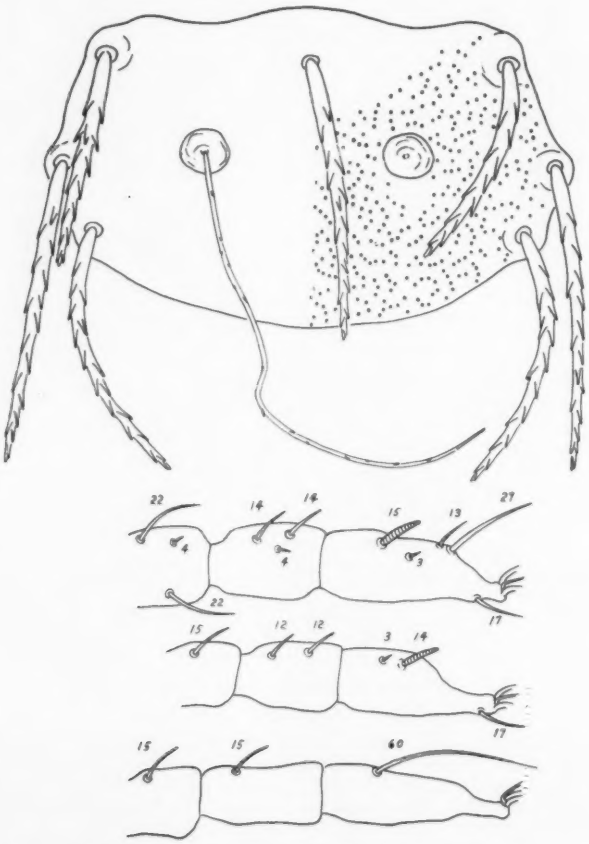

FIGURE 7. *Hoffmannina handleyi* n. sp. Scutum and specialized setae of legs.

Polylopadium kramisi new species

(Figures 8, 9)

Type data: Holotype and 21 paratypes, RML No. 35267, off *Liomys adspersus,* Summit Gardens, Canal Zone, 21 September 1954; 18 paratypes, same host, Curundu, C. Z., 8 July 1954; 8 paratypes, same host, National Forest, C. Z., 21 December 1954; 1 paratype off *Proechimys semispinosus,* location not given, 7 January 1954; Gordon Field, collector.

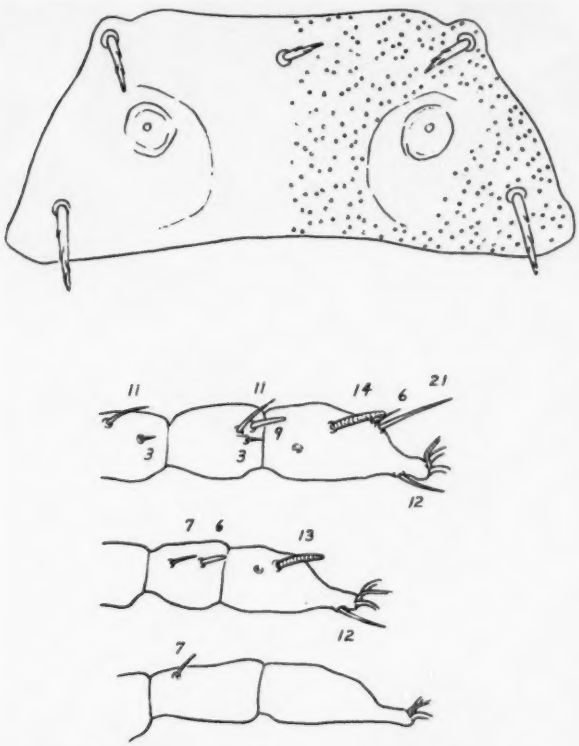

FIGURE 8. *Polylopadium kramisi* n. sp. Scutum and specialized setae of legs.

Holotype, RML; paratypes RML, USNM, BM, CNHM, SAM.

Other material: Two specimens, RML No. 35868, off *Peromyscus* sp., Chiriqui, Panama, 12 February 1960, Vernon J. Tipton, collector. These, while not specifically distinct from the type series, may represent a subspecies, and are distinguished by about 40 ventral setae and minutely barbed scutal and dorsal setae.

Diagnosis: Trapezoidal scutum with very short setae, 13 punctate dorsal platelets, 1 genuala I, no genualae II or III, short and pointed dorsal setae.

Body: Ovate, caudal margin truncate to emarginate. Eyes absent. Dorsum with 13 punctate platelets, devoid of setae, arranged as figured. Length and width of holotype, partly engorged, 280 by 185. Anus at fifth row of ventral setae.

Gnathosoma: Moderately punctate. Blades with minute tricuspid cap and a subapical ventral notch. Palpal setae B/B/BBB, the branches delicate and few; claw trifurcate; tarsus with 5 branched setae and a tarsala. Galeal seta nude.

Scutum: Trapezoidal and punctate as figured. Setae short, pointed, transversely striate, and with vestigial barbs. Sensillae broken off throughout the series, however, an occasional stump suggests sensillae of the expanded type. Measurements of holotype: AW, 56; PW, 70; SB, 40; ASB, 19; PSB, 18; AP, 23; AM, 6; AL, 8; PL, 12; S, . . .

Legs: Punctate. Specialized setae as figured. Nonspecialized setae with sparse, short branches.

Body setae: Dorsal setae similar to scutals, 19 to 24 long, arranged 4-4-2-4-4-2. Ventral setae 2-2 sternals plus about 20, postanals similar to dorsals, preanals smaller and with more pronounced barbs.

Named for N. J. Kramis, staff photographer, Rocky Mountain Laboratory.

FIGURE 9. Dorsum of *Polylopadium kramisi* n. sp. showing scutum and platelets, 645×. Oblique lighting photomicrograph by N. J. Kramis.

Trombicula arremonops new species

(Figure 10)

Type data: Holotype, RML No. 35280, off bird, *Arremonops conirostris,* Coco Plantation, Panama, 4 May 1955, Gordon Field, collector. In the Rocky Mountain Laboratory.

Diagnosis: A mastitarsala III, 3 genualae I, deep scutum, galeal seta branched, long legs, long setae.

Body: Ovate. Eyes large, 2/2, in a plate. Length and width of holotype, unengorged, 278 by 154. Anus at third row of ventral setae.

Gnathosoma: Densely and conspicuously punctate. Blades with small subapical dorsal tooth and a larger ventral tooth. Galeal seta branched. Palpal setae long, B/B/NNB; claw trifurcate; tarsus with 7 branched setae, a subterminala and a long tarsala.

Scutum: As figured. Measurements of holotype: AW, 85; PW, 87; SB, 45; ASB, 32; PSB, 45; AP, 33; AM, 53; AL, 39; PL, 60; S, 70.

Legs: Conspicuously punctate. Specialized setae as figured. Non-specialized setae long, sparsely to densely branched.

Body setae: Dorsals long, with appressed barbs, like scutals, 55 to 60 long, arranged 2-6-6-4-2. Ventral setae 2-2 sternals plus 12, postanals similar to dorsals.

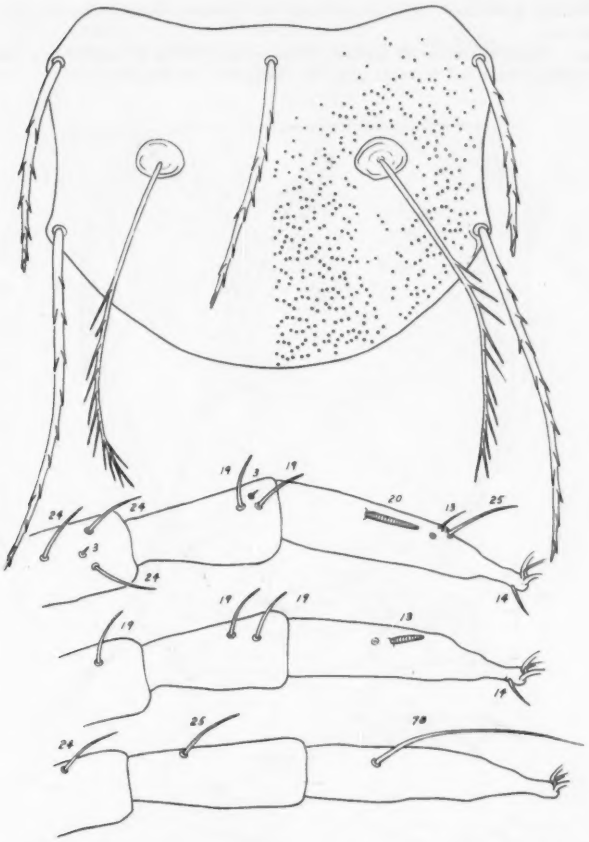

FIGURE 10. *Trombicula arremonops* n. sp. Scutum and specialized leg setae.

Trombicula caccabulus new species

(Figure 11)

Type data: Holotype and a paratype, RML No. 35875, off *Peromyscus* sp., Chiriqui, Panama, 14 February 1960; 42 paratypes off *Peromyscus nudipes, Peromyscus* sp., *Oryzomys fulvescens, Oryzomys* sp., *Reithrodontomys mexicanus,* and *Scotinomys teguina,* Chiriqui, 30 January to 4 May 1960; Vernon J. Tipton and C. Marvin Keenan, collectors.

Holotype, RML; paratypes, RML, USNM, CNHM, BM, SAM.

Diagnosis: Scutum deep, with thick setae, a mastitarsala III, 3 genualae I, dorsal formula begins 2–6.

Body: Ovate. Eyes large, anterior and posterior about equal size, 2/2, in a plate. Length and width of holotype, unengorged, 290 by 185. Anus at third row of ventral setae.

Gnathosoma: Densely and conspicuously punctate. Blades with tricuspid cap. Galeal seta nude. Palpal setae B/B/NNB; claw trifurcate; tarsus with 7 branched setae, a subterminala and a tarsala.

Scutum: As figured, with unusually thick setae and broadly rounded posterior margin. Measurements of holotype: AW, 79; PW, 98; SB, 36; ASB, 29; PSB, 36; AP, 24; AM, 55; AL, 52; PL, 88; S, 100.

Legs: Densely punctate. Specialized setae as figured. Nonspecialized setae moderately branched to plumose.

Body setae: Dorsals similar to scutals, 50 to 68, decreasing in length posteriorly, arranged 2-6-6-4-2-2. Ventral setae 2-2 sternals plus 18, postanals like dorsals.

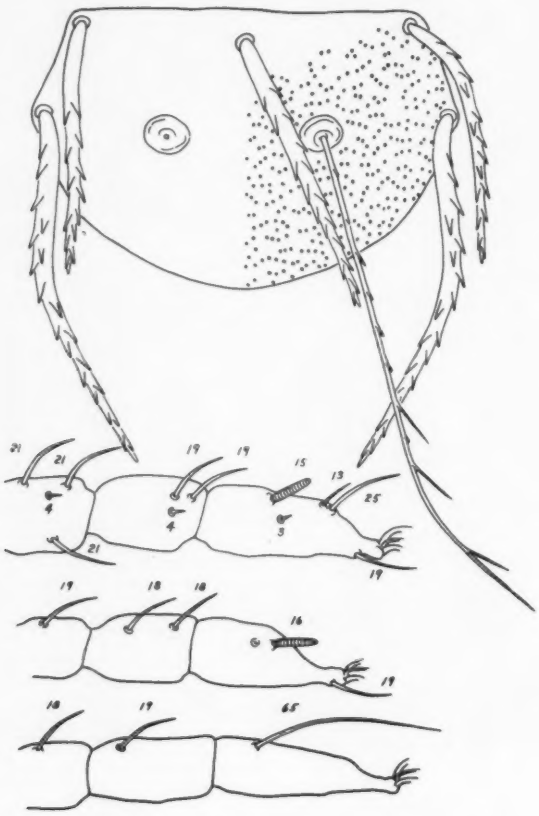

FIGURE 11. *Trombicula caccabulus* n. sp. Scutum and specialized leg setae.

Trombicula chiriquensis new species

(Figure 12)

Type data: Holotype and 2 paratypes, RML No. 35966, off *Scotinomys teguina*, Chiriqui, Panama, 4 May 1960; 16 paratypes, same host, *Peromyscus* sp., *Reithrodontomys* sp., Chiriqui, 26 January to 4 May 1960; Vernon J. Tipton and C. Marvin Keenan, collectors.

Holotype, RML; paratypes, RML, USNM, CNHM, BM, SAM.

Diagnosis: A mastitarsala III, 3 genualae I, deep scutum. Separated from *T. caccabulus* n. sp. by the more sparsely punctate scutum, more slender and longer branched scutal setae, and branched palpal laterotibial seta.

Body: Ovate. Eyes large, 2/2, in a plate. Length and width of holotype, unengorged, 280 by 185. Anus at fourth row of ventral setae.

Gnathosoma: Punctate. Blades with tricuspid cap. Galeal seta nude. Palpal setae B/B/NBB; claw trifurcate; tarsus with 7 branched setae, a subterminala and a tarsala.

Scutum: As figured. Measurements of holotype: AW, 68; PW, 84; SB, 31; ASB, 29; PSB, 26; AP, 23; AM, 35; AL, 42; PL, 52; S, 63.

Legs: Conspicuously punctate. Specialized setae as figured. Nonspecialized setae moderately to densely branched.

Body setae: Dorsals like scutals, 40 to 46 long, arranged 2-6-6-6-4-2-2. Ventral setae 2-2 sternals plus 18, postanals similar to dorsals.

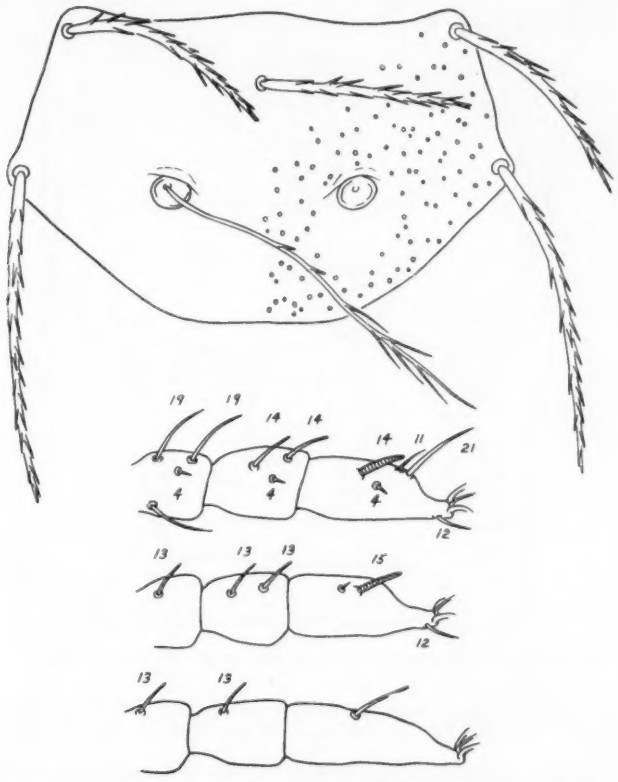

FIGURE 12. *Trombicula chiriquensis* n. sp. Scutum and specialized setae of legs.

Trombicula cribanus new species

(Figure 13)

Type data: Holotype, RML No. 35306, off *Proechimys semispinosus* (spiny rat), Cerro Azul, Panama, 24 January 1956, Gordon Field, collector. In the Rocky Mountain Laboratory.

Diagnosis: A mastitarsala III, 2 genualae I, dorsal formula begins 2-8.

Body: Ovate. Eyes 2/2, in a plate. Length and width of holotype, partly engorged, 473 by 350. Anus at fourth row of ventral setae.

Gnathosoma: Conspicuously punctate. Blades with tricuspid cap. Galeal seta nude. Palpal setae B/B/NNB; claw trifurcate; tarsus with 7 branched setae, a subterminala and a tarsala.

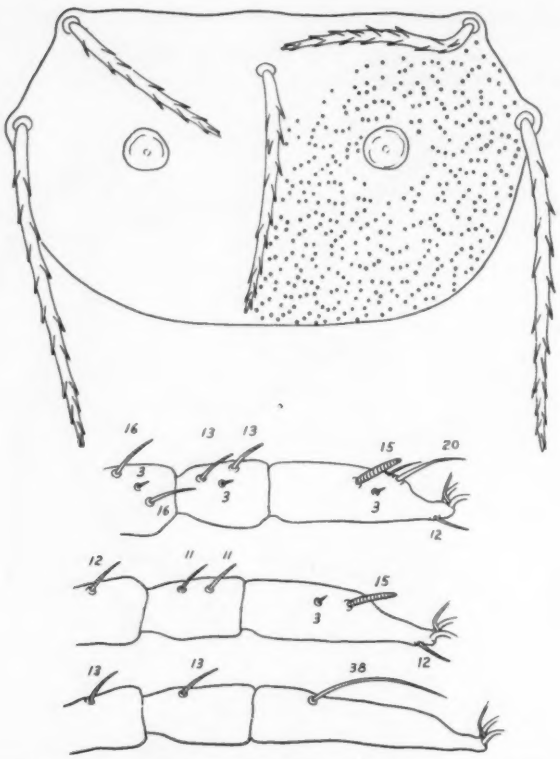

FIGURE 13. *Trombicula cribanus* n. sp. Scutum and specialized setae of legs.

Scutum: As figured, fairly deep, posterior margin flattened, PL's near AL's. Measurements of holotype: AW, 66; PW, 85; SB, 41; ASB, 24; PSB, 30; AP, 18; AM, 48; AL, 34; PL, 54; S, . . .

Legs: Punctate. Specialized setae as figured. Nonspecialized setae sparsely to moderately barbed.

Body setae: Dorsals similar to scutals, 37 to 52, increasing in length laterally, arranged 2-8-6-6-4-2. Ventral setae 2-2 sternals plus 24, postanals like dorsals.

Trombicula dicrura new species

(Figure 14)

Type data: Holotype and 6 paratypes, RML No. 35938, off *Peromyscus nudipes,* Chiriqui, Panama, 14 February 1960; 113 paratypes, same host, *Peromyscus* sp., *Oryzomys* sp., *Heteromys desmarestianus, Scotinomys teguina, Sciurus granatensis,* and *Sciurus* sp., Chiriqui, 30 January to 4 May 1960; Vernon J. Tipton and C. Marvin Keenan, collectors, 1 paratype off *Proechimys semispinosus,* Bocas del Toro, Panama, 22 January 1960.

Holotype, RML; paratypes RML, USNM, CNHM, BM, SAM.

Diagnosis: Palpal tibial claw bifurcate, scutum subrectangular with sinuous anterior margin, a mastitarsala III, 2 genualae I, parasubterminala branched.

Body: Ovate. Eyes large, 2/2 in a plate. Anus at third row of ventral setae. Length and width of holotype, unengorged, 288 by 185.

Gnathosoma: Densely and conspicuously punctate. Cheliceral blades with subapical dorsal tooth, larger ventral tooth and subapical ventral notch. Galeal seta nude. Palpal setae B/B/NNB, the laterotibial occasionally forked or branched; claw bifurcate, the prongs of nearly equal length; tarsus with 6 branched setae, a subterminala and a tarsala.

Scutum: As figured, conspicuously punctate, subrectangular with sinuous anterior and mildly convex posterior margins. Measurements of holotype: AW, 77; PW, 84; SB, 37; ASB, 32; PSB, 22; AP, 29; AM, 59; AL, 55; PL, 72; S, 75.

Legs: Conspicuously punctate. Specialized setae as figured, parasubterminala forked or branched, occasional specimens with 3 genualae I on one leg. Nonspecialized setae moderately branched to plumose.

Body setae: Dorsals similar to scutals, 68 to 72, arranged usually 2-6-6-6-2-2. Ventrals 2-2 plus 12, postanals like dorsals.

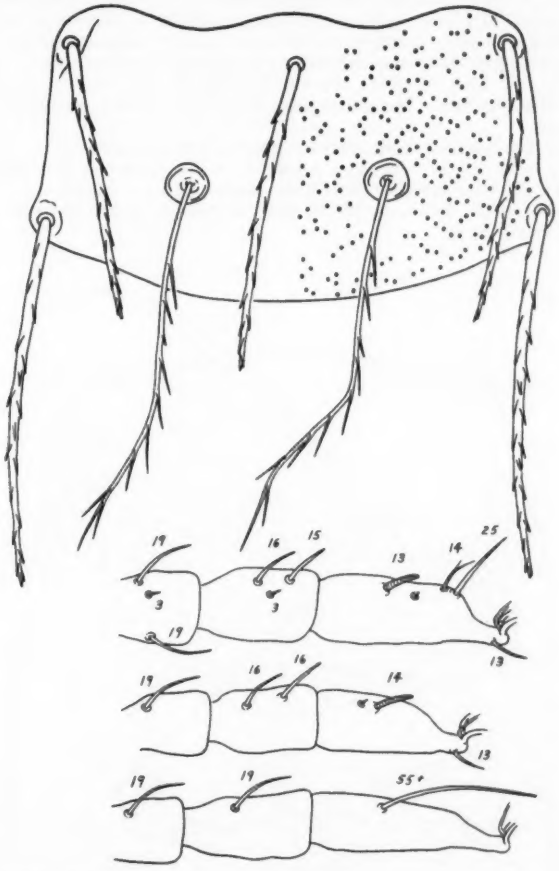

FIGURE 14. *Trombicula dicrura* n. sp. Scutum and specialized setae of legs.

Trombicula keenani new species

(Figure 15)

Type data: Holotype and a paratype, RML No. 35934, off *Peromyscus nudipes,* Chiriqui, Panama, 13 February 1960; 71 paratypes off same host, *Peromyscus* sp., *Oryzomys fulvescens, Oryzomys* sp., *Scotinomys teguina, Reithrodontomys mexicanus, Sciurus granatensis,* and *Sciurus* sp., Chiriqui, 30 January to 4 May 1960; Vernon J. Tipton and C. Marvin Keenan, collectors.

Holotype, RML; paratypes, RML, USNM, CNHM, BM, SAM.

Other material: Ten specimens off *Proechimys semispinosus* and *Hoplomys gymnurus*, Camp Piña and National Forest, Canal Zone, 10 March and 12 December 1954, 26 January to 3 February 1955, Gordon Field, collector.

This form differs from typical *T. keenani* by having 2 instead of 4 humeral setae.

Diagnosis: Dorsal formula begins 4-4 or 2-4, deep scutum, a mastitarsala III, 3 genualae I.

Body: Ovate. Eyes large, 2/2 in a plate, anterior and posterior about equal size. Anus at about fourth row of ventral setae. Length and width of holotype, unengorged, 320 by 215.

Gnathosoma: Densely punctate. Blades with minute subapical dorsal tooth and larger ventral tooth. Galeal seta long, nude. Palpal setae B/B/NNB, dorso- and laterotibial sometimes forked; claw trifurcate; tarsus with 7 branched setae, a subterminala and a tarsala.

Scutum: As figured, very deep and densely punctate. Measurements of holotype: AW, 82; PW, 103; SB, 43; ASB, 34; PSB, 40; AP, 24; AM, 45; AL, 45; PL, 83; S, 105.

Legs: Densely punctate. Specialized setae as figured. Nonspecialized setae moderately barbed to plumose.

Body setae: Dorsals thick, with short stout barbs, like scutals, fairly uniform length (60), arranged usually 4-4-4-2-2, or sometimes 2-4-4-2-2; a few specimens with additional irregularly placed setae. Ventral setae 2-2 plus 18, postanals similar to dorsals.

Named for one of the collectors, Mr. C. Marvin Keenan, United States Army Caribbean, Fort Clayton, Canal Zone.

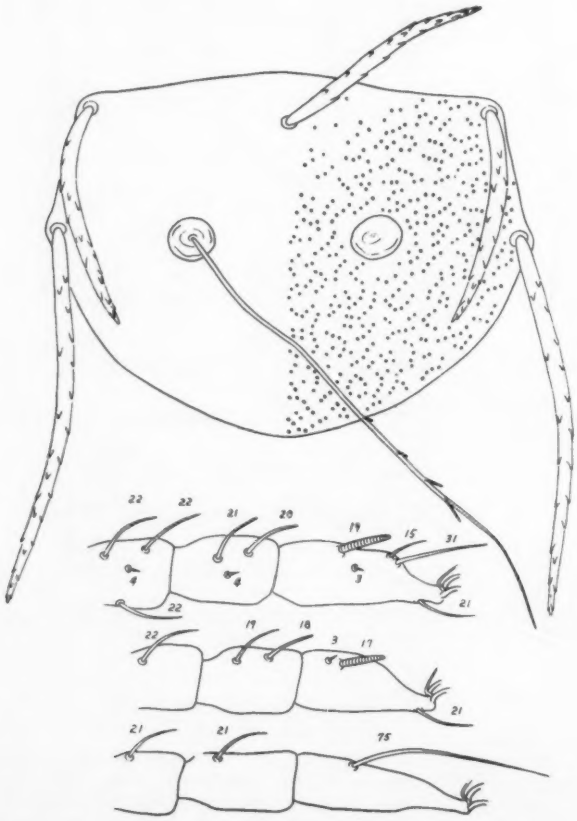

FIGURE 15. *Trombicula keenani* n. sp. Scutum and specialized setae of legs.

Trombicula liomys new species

(Figure 16)

Type data: Holotype, RML No. 35292, off *Liomys adspersus,* Curundu, Canal Zone, 8 July 1954; 3 paratypes, same host, Corozal, C. Z., 21 October 1954; Gordon Field, collector. Holotype, RML; paratypes, RML, USNM.

Diagnosis: Scutum rectangular with sinuous posterior margin, 3 pairs sternal setae, no mastitarsala III, 2 genualae I, dorsal formula begins 2-2-6. Because sensillae are missing from all specimens of the series, generic assignment is in question.

Body: Ellipsoidal. Eyes 2/2 in a plate. Length and width of holotype, partly engorged, 556 by 350. Anus at seventh row of ventral setae.

Gnathosoma: Densely punctate. Blades with prominent tricuspid cap and a subapical dorsal hook. Galeal seta nude. Palpal setae B/N/BNB; claw trifurcate; tarsus with apparently 6 branched setae, a subterminala and a tarsala.

Scutum: As figured. Measurements of holotype: AW, 74; PW, 85; SB, 42; ASB, 33; PSB, 23; AP, 41; AM, 40; AL, 32; PL, 48; S, . . .

Legs: Punctate. Specialized setae as figured, microgenuala and microtibiala I long. Nonspecialized setae sparsely branched.

Body setae: Dorsals similar to scutals, 44 to 47, arranged 2-2-6-6-6-4-4-2. Ventral setae 2-2-2 sternals plus about 64 postanals similar to dorsals.

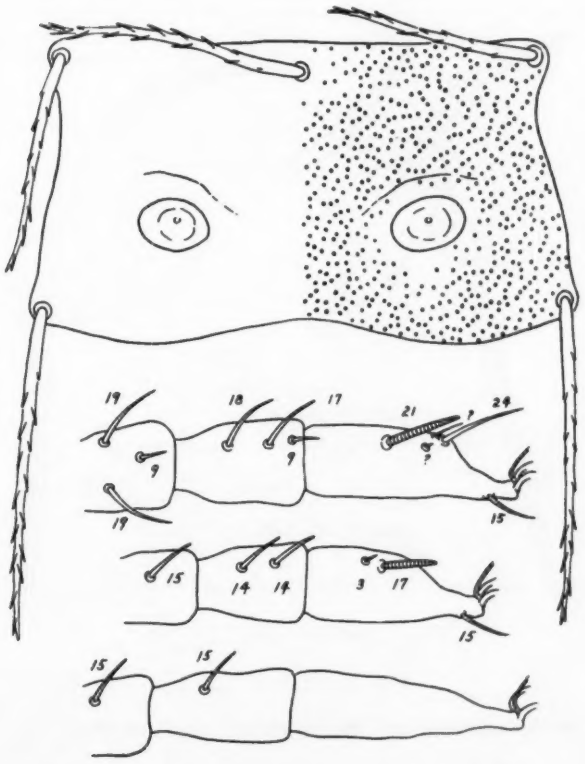

FIGURE 16. *Trombicula liomys* n. sp. Scutum and specialized leg setae.

Trombicula tiptoni new species

(Figure 17)

Type data: Holotype, RML No. 35930, off *Peromyscus nudipes*, Chiriqui, Panama, 12 February 1960, Vernon J. Tipton, collector. In the Rocky Mountain Laboratory.

Diagnosis: All body setae (humerals, dorsals, sternals, preanals and postanals), scutal setae, and nonspecialized leg setae of similar form, i.e., plumose with long coarse branches. Two genualae I, no mastitarsala III. Femoral and genual palpal setae nude. Sensillae branched.

Body: Quadrate. Eyes 2/2, no plate. Anus at about sixth row of ventral setae. Length and width of holotype, engorged, 1050 by 825.

Gnathosoma: Densely punctate. Blades with tricuspid cap. Palpal setae N/N/NNB, long; claw trifurcate; tarsus with 7 branched setae, a subterminala and a tarsala. Galeal seta plumose.

Scutum: As figured, conspicuously punctate, SB posterior to PL's, sensillae branched. Measurements of holotype: AW, 74; PW, 102; SB, 32; ASB, 41; PSB, 19; AP, 27; AM, 55; AL, 47; PL, 84; S, 73.

Legs: Densely punctate. Specialized setae as figured. Nonspecialized setae plumose, similar in form to scutal and body setae.

Body setae: Dorsal setae 2 humerals plus about 64, similar to scutals and ventrals, 40 to 80, decreasing in length posteriorly. Ventral setae 2-2 sternals plus more than 100, the preanals begin at second pair of sternals and are not structurally differentiated from the postanals.

Named for the collector, Captain Vernon J. Tipton, United States Army Caribbean, Fort Clayton, Canal Zone.

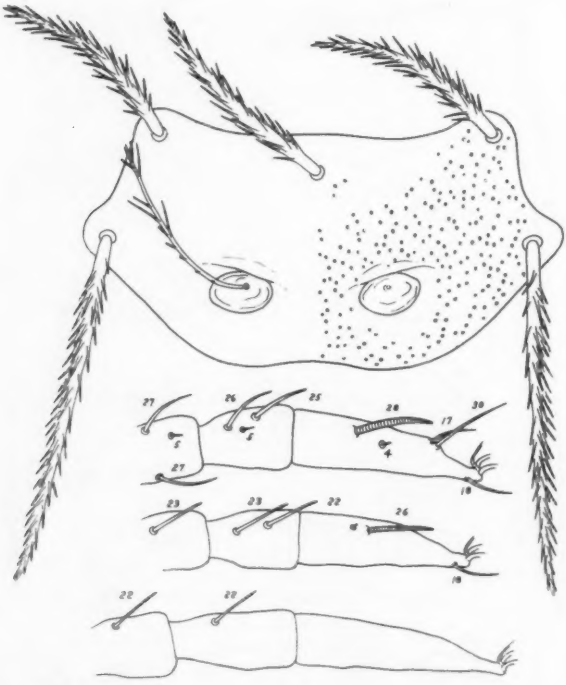

FIGURE 17. *Trombicula tiptoni* n. sp. Scutum and specialized leg setae.

Vanidicus new genus

Trombiculine larvae superficially like *Pseudoschöngastia*. Cheliceral blades with tricuspid cap. Palpal tarsus with 5 branched setae and a tarsala. Eyes present. Posterolateral setae off scutum. Leg segmentation 7-6-6. Parasubterminala lacking. No ventral humeral setae between coxae II and III.

Type species: *Vanidicus tricosus* new species.

Vanidicus tricosus new species

(Figure 18)

Type data: Holotype and 2 paratypes, RML No. 35292, off *Liomys adspersus*, Curundu, Canal Zone, 8 July 1954; 1 paratype, same host, Summit Gardens, C. Z., 21 September 1954; Gordon Field, collector.

Holotype, RML; paratypes, RML and USNM.

Diagnosis: Three genualae I, parasubterminala absent, dorsal formula begins 2-6.

Body: Ellipsoidal. Eyes 22, apparently no plate. Length and width of holotype, nearly engorged, 330 by 240. Anus at fifth row of ventral setae.

Gnathosoma: Moderately punctate. Blades with tricuspid cap. Palpal setae B/N/NNN; claw trifurcate; tarsus with 5 branched setae and a tarsala. Galeal seta nude.

Scutum: As figured, sparsely punctate, sensillae broken off all specimens, but remaining stumps suggest expanded heads. Measurements of holotype: AW, 45; SB, 24; ASB, 18; PSB, 16; AM, . . .; AL, 14; PL, 30; S, . . .

Legs: Moderately punctate. Specialized setae as figured. No parasubterminala. Non-specialized setae sparsely branched.

Body setae: Dorsal setae like posterolaterals, 22 to 30, decreasing in length, posteriorly, arranged 2-6-6-4-4-2. Ventral setae 2-2 sternals plus 28, postanals similar to dorsals.

V. tricosus differs from species of *Pseudoschöngastia* by lacking a parasubterminala and ventral humeral setae, and by the dorsal formula. Species of *Pseudoschöngastia* have 2 pairs of humeral setae and fairly numerous dorsal setae.

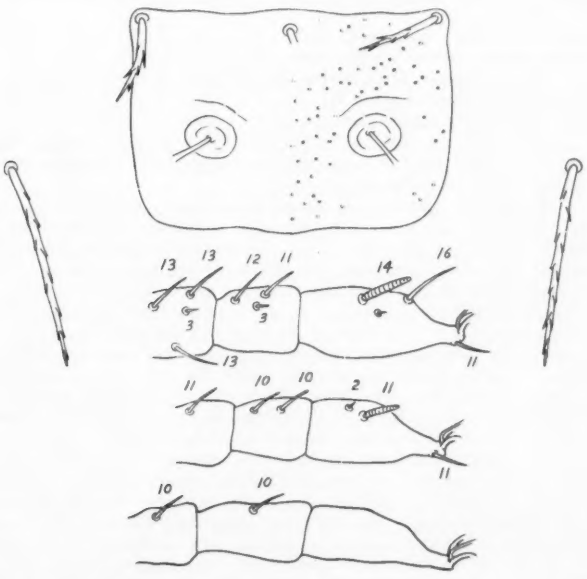

FIGURE 18. *Vanidicus tricosus* n. sp. Scutum and specialized setae of legs.

SUMMARY

Seventeen new species of chiggers and three new genera, *Aniatrus, Polylopadium,* and *Vanidicus,* are described from Panama and the Canal Zone. New species and their type hosts are: *Euschöngastia spissa, Hoffmannina handleyi, Trombicula caccabulus, T. dicrura, T. keenani,* and *T. tiptoni* off *Peromyscus nudipes; Ascoschöngastia dyscrita, Polylopadium kramisi, T. liomys* and *Vanidicus tricosus* off *Liomys adspersus; Odontacarus fieldi* off *Zygodontomys cherriei; Aniatrus bifax* off *Dasypus novemcinctus; Eus. cunctata* off *Oryzomys talamancae; Eus. tragulata* off *Nasua narica; T. arremonops* off *Arremonops conirostris; T. chiriquensis* off *Scotinomys teguina; T. cribanus* off *Proechimys semispinosus.*

The genus *Polylopadium* is unique among chiggers in that the larvae have 13 dorsal plates in addition to the scutum.

RESEARCH NOTE

PARASITIC MITES ON MARMOTS IN UTAH.

This note on mites found on yellow-bellied marmots, *Marmota flaviventris* ssp., is one of a series reporting the results of a survey being made of the parasitic arthropods found on vertebrates of Utah (Beck, 1955, Brigham Young Univ. Sci .Bull. 1(1): 1–64; Allred, Beck and White, 1960, Brigham Young Univ. Sci. Bull. 1 (4): 1–42).

Fifty-three marmots were collected over an 11-year period from 24 areas distributed in 14 counties during the months of April through August. Following are records of the mites found on marmots from the eastern part of the Great Basin and the western part of the Colorado Basin in Utah.

Marmota flaviventris engelhardti Allen
Euschongastia luteodema Brennan
Delano Ranger Sta., Beaver Co., 25 June 1957, 97 larvae
Marmota flaviventris nosophora Howell
Haemolaelaps glasgowi (Ewing)
Bald Mtn., Summit Co., 24 August 1956, 4 females, 1 deutonymph
Pole Creek Mtn., Duchesne Co., 24 August 1957, 2 females, 1 protonymph
Trombicula microti Ewing
Timpooneke Ranger Sta., Utah Co., 8 August 1958, 6 larvae.

Mites were found only on five of the marmots examined. This incidence is low when compared with the finding of fleas on 37 of the marmots, lice on 25, and ticks on 14. Only six apparently lacked ectoparasites. All infested hosts were collected at elevations between 7,000 ft and 10,000 ft. None of the marmots apparently was heavily infested with mites except one specimen from the Delano Ranger Station which possessed 97 chiggers. Not more than six mites were found on any one of the other infested animals.

There are relatively few published records of mites from marmots, although there are numerous records of mites found on animals which live in close association with them. Wharton and Fuller (1952, Ent. Soc Wash. Mem. No. 4) recorded several hosts for the chigger mite *Trombicula microti,* namely *Glaucomys, Microtus, Ochotona, Tamiasciurus* and *Zapus,* all of which frequently live near marmot habitats in some areas of high elevation. Wharton and Fuller (ibid.) listed *M. flaviventer nosophora* as host for the chigger mite *Euschongastia luteodema.* Brennan and Beck (1955, Great Basin Nat. 15: 1–26) listed *Ochotona, Microtus* and *Zapus* as hosts for *T. microti,* and *M. flaviventer* as host for *T. harperi* in Utah. Strandtmann and Wharton (1958, Univ. Md. Inst. Acarology Contrib. No. 4) suggested that *Haemolaelaps glasgowi* is truly cosmopolitan with no host preference. They listed *M. monax* and *M. sibirica* as two of its hosts. It is likely that other species of mites will be found on marmots in Utah when additional animals are examined.—DORALD M. ALLRED, *Department of Zoology and Entomology, Brigham Young University, Provo, Utah.*

STUDIES OF THE PHILOPTERIDAE (MALLOPHAGA) FROM BIRDS OF THE ORDER PROCELLARIFORMES
1. THE GENUS *HALIPEURUS* THOMPSON

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The Mallophaga of the birds of the order Procellariformes have attracted the interest of entomologists since the time of Fabricius. My own study was initiated when I had the good fortune to acquire a collection of these insects from the American Museum of Natural History. That material, plus an even larger amount from other sources, totaling well over 2,000 specimens, made it possible to revise the generic concepts of the lice of the family *Philopteridae* found on the procellariforms and to revise the sizeable subgenus *Halipeurus*.

This paper revising the genus *Halipeurus* is the first part of several concerned with the lice of the procellariform birds. This paper and the succeeding papers are based in part or in whole on my thesis, submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology of Harvard University. The papers to follow will be concerned with revisions of the other genera of the family Philopteridae. The final paper in this series will discuss the degree of concomitant phylogeny exhibited between these parasites and their hosts.

METHODS

To be properly studied the lice must be cleared and mounted on microscope slides. The usual technique of clearing in caustic potash and mounting in Canada Balsam is satisfactory but time consuming. A new technique, described by Salmon (1951), using a polyvinyl alcohol, lactic acid, and phenol compound, is much more satisfactory, requires considerably less time, and makes it possible to see the chaetotaxy and subtleties of sclerotization with greater clarity. With a phase microscope, specimens mounted in the polyvinyl alcohol medium show much internal structure formerly destroyed by using KOH. Among the disadvantages of using polyvinyl alcohol is that it does not completely clear the darkly colored material within the alimentary tract, the slides must be examined periodically to fill in the vacuities that occasionally appear under the coverslip, and the medium occasionally hardens with a dispersion of small crystal-like objects in it.

The lice are placed directly in the mixture, regardless of the preservative used. Even dry specimens are satisfactorily re-expanded, although best results are obtained when such material is first soaked a few days in 30 percent alcohol. The various possible solutions are all discussed in Salmon's paper (op. cit.), the most successful mixtures seem to be those with the higher percentages of phenol.

The technique, in addition to clearing well, leaves the general pigmentation more or less unchanged. This is particularly useful in the case of species that have unusually heavy sclerotization.

It is standard procedure on the part of many workers to remove the male genitalia from the lice. In a properly cleared slide this is often not necessary. The genitalia are sufficiently visible when left within the insect to serve their diagnostic purpose, especially since the terminal segments are of such value that the possibility of destroying them outweighs the advantages of removing the genitalia. Removing the genitalia, of course, serves its purpose when more exacting study of the genitalia is required.

Lice may be removed from a bird study skin without damage to the skin if it is in good condition. A blunt dissecting needle thrust under the feathers and then moved out at right angles

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to the shaft of the feathers while lifting them slightly was found to be most effective. If large numbers of lice are on the bird, tapping will dislodge many of them. Lice so taken from skins are often perfect specimens. They tend to be shaken out over a period of years and thus fall to the bottom of the drawer in which the skins are stored. If, subsequently, another bird is placed upon the louse, it may adhere to this bird. Consequently, unless a bird is found with more than a few lice of one species, some doubt exists concerning the true host. One should keep in mind also that the original collector of the bird may not have kept his freshly collected specimens separated from each other, and thus allowed some of the lice to move about from one host to another before they died.

The drawings were made with the aid of a camera lucida attached conventionally to a compound microscope. A low-power ocular (2×) was used in conjunction with the high power (44×) objective for all drawings in order to keep them at the same scale and sufficiently detailed to cover the wide size range of these lice.

Since the ventral chaetotaxy of the terminal segments was of primary interest, the insects were usually mounted ventral side up on the slide. These species may be determined with a binocular dissecting microscope, providing a magnification of 120× is possible. Using such a microscope, however, these drawings are oriented correctly only if the insects are mounted ventral side down. The method of mounting actually is a matter of choice, and becomes important only when species with peculiar asymmetrical developments are concerned.

The measurements were made with the aid of an ocular micrometer. Since any mounting medium tends to distort the specimens, the measurements vary in reliability. Because of the more or less inflexible structure of the head, those measurements concerned with its length and breadth are very accurate. Total length and abdomen length are less so, while abdomen width is unreliable unless obviously undistorted specimens are used.

The head ratio and abdomen ratio are computed by dividing the length of each by the greatest breadth.

The following abbreviations are used in the text:

(AMNH) American Museum of Natural History, New York City;
(BMNH) British Museum (Natural History), London;
(GBT) Referring to the collection of Gordon B. Thompson;
(Meinertzhagen) Referring to the Meinertzhagen collection of Mallophaga, in the British Museum of Natural History;
(RLE) Material collected personally, as indicated; and
NV No variation within reasonable limits of measurement.

The host names used are those in Peters' *Check List of Birds of the World*, **1**, 1931, unless otherwise noted in the text. All drawings appearing in this paper refer to the genus *Halipeurus*.

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The bulk of the material herein discussed was collected personally from museum skins. Mr. James E. Peters, Curator of Birds, Museum of Comparative Zoology, Harvard University, kindly gave me access to the collections of birds. Mr. Charles O'Brien, of the Department of Ornithology, American Museum of Natural History, made collections available to me several times during the past few years.

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The type material resulting from this study has been distributed in such a manner as to give the British Museum (Natural History), the American Museum of Natural History, and the Museum of Comparative Zoology each as complete a set of types and/or paratypes as possible. Additional type material will be deposited in the United States National Museum.

I. BIOLOGY AND ECOLOGY

Ecologically, many of these lice are strongly adapted to restricted niches, i.e., to various localized parts of their bird hosts. Clay (1949) has discussed this aspect particularly well. The elongate lice treated in detail here are adapted to live on the back and wings of the birds. To gain some idea of the ecological diversity of these lice, however, consider the albatross, *Diomedea,* on which the following genera may be found, usually as follows: *Harrisoniella* on the primary wing feathers, *Episbates* on the secondary wing feathers, *Naubates* and *Perineus* on the back and proximally on the wing, *Docophoroides* on the head and neck, and *Austromenopon* (*Menoponidae*) under the wings, and on the breast and belly.

These lice exist in a relatively uniform environment, with a high constant temperature (that of their host) and an abundant food supply (feathers). Their principal enemy seems to be the host. Although they move quickly through the feathers, they must occasionally be brushed off or crushed by the bird's beak.

Transfer from one host to another is presumably largely direct. Mainly, it probably consists of movement from the adult to young, although the lice may migrate at any time that birds come in close contact with one another. It is well known that the *Hippoboscidae* carry Mallophaga about, and while these flies are not known to frequent the procellariform birds, the possibility of that type of transfer exists.

The material at hand indicates that there are only three instars, as in other genera of Mallophaga that have been studied. The length of the period of development from egg to adult is probably about one month; Wilson (1934, 1939) has data on two species infesting chickens which indicates that each instar has a duration of approximately 1 week. Unfortunately, it is almost impossible to obtain living lice of the genera treated here, as the procellariform birds are found typically about the equatorial region and further south. The few species found in the northern hemisphere do not commonly visit the shores of the New England region except under unusual conditions, although small colonies of the storm petrel, *Hydrobates p. pelagicus* (Linn), are known to nest on a few islands off the coast of eastern North America.

Order MALLOPHAGA *Nitzsch*
Suborder ISCHNOCERA *Kellogg*
Family Philopteridae *Burmeister*
Tribe Philoceanini *tribe nov.*

Description: Relatively elongate Philopteridae with broad abdominal buttresses having (usually) well developed anterior and/or posterior medial processes; abdominal tergites sclerotized completely across dorsum, not divided medially in either sex (*Harrisoniella* excepted, with slight medial division); signature not divided medially but sometimes with a gutta, well developed to completely subordinated; genitalia simple, typically asymmetrical, parameres usually subequal and only lightly sclerotized; without complex setal patterns on terminal segments.

Key to the Complexes and Genera of Philoceanini

1. With relatively small, rounded medial processes; signature usually longer than broad, sometimes with a gutta ..... (*Philoceanus*-complex) 2
   With relatively bulbous or elongate medial processes; signature usually broader than long, never with gutta, usually considerably reduced ..... (*Pseudonirmus*-complex) 3
2. Very slender lice, 8 or more times as long as broad ..... *Halipeurus*
   Less than 7 times as long as broad ..... *Philoceanus*
3. Very large lice, (5–11 mm), basal segment of male antenna with elongate process ..... *Harrisoniella*
   Not so large 4
4. Cuticle with obvious 'pebbled' appearance ..... *Episbates*
   Cuticle not as above, may be either darkly pigmented overall or with buttresses only colored ..... *Pseudonirmus*

The *Philoceanus*-complex includes the genera *Philoceanus* and *Halipeurus*, each of which is further divided into subgenera and species-groups. The complex occurs on almost all of the procellariforms, with the possible exception of the albatrosses (*Diomedea* and *Phoebetria*). All the species are well colored. The medial processes are usually well developed but not knobbed, elongate, or bulbous. They are all generally heavily sclerotized, and the mid-abdominal segments of the males typically have a heavily sclerotized band across the tergite that is strongly narrowed medially. The signature is well developed and as long or longer than broad. All of the lice are relatively slender and thereby adapted to live on the wings and backs in particular of their hosts.

The Genus Halipeurus *Thompson*

The genus *Halipeurus* herein revised in part comprises a group of slender, mahogany-colored bird lice of moderate size, 3 to 5 millimeters long. They are typically found on birds of the families Procellaridae, Pelecanoididae, and Hydrobatidae, the shearwaters and petrels, the diving petrels, and the storm petrels, respectively. They are usually found on the backs and wings of those birds, being functionally adapted to these areas.

These lice have attracted the interest of several workers in the past 50 years, particularly Launcelot Harrison of the University of Sydney, Australia. Harrison died before he had published much of his findings, the posthumous publishing of a manuscript (1937) revealing that he had accomplished far more than was indicated in this last paper. There are specimens in existence that he labeled, additional evidence that he did have a clear idea of the species involved although he never published their descriptions. Recently, Mr. Gordon B. Thompson has continued research on these lice, and he has published many papers on them. It was necessary that I make arrangements with him to divide this work. At that time I did not have a clear idea of the validity of the various genera involved and it was agreed that I confine my work to the genus *Halipeurus* of which I had a large collection, and that I would leave the genera *Synnautes* and *Naubates* to him. Since that time it has become abundantly clear that *Synnautes* as a taxon would rank no higher than a subgenus, pending more material, and it is so considered in this work. I have not

described any new typical *Synnautes,* but have taken the liberty of describing one atypical species that is pertinent to the content of this study.

MORPHOLOGY

The homologies of the various parts of the mallophagan head, long debated, were competently investigated by Symmons (1952). This study and that of Clay (1951) have resulted in a considerably different understanding of the morphology and terminology of the head. These two papers should be referred to by anyone interested in these matters. The terminology used in this paper is for the most part that advocated by Clay (op. cit.).

Head: The head is roughly twice as long as broad, in subgenus *Synnautes* slightly narrower and with straighter lateral margins than in *Halipeurus* (see fig. 2, A and B). It is buttressed laterally, as in the thorax and abdomen, with a heavily sclerotized, internally concave bar. This buttress is made up of three parts: the first, the premarginal carina, on either side of the ante-clypeus, giving support to the ante-clypeus; the second, anterior to the antennal socket, the postmarginal carina; and the last, that of the lateral margin of the parietal area, the marginal temporal carina. Anteriorly, the postmarginal carina arcs medially and then posteriorly on the dorsal surface of the head in the form of a heavily sclerotized band, the dorsal carina. In *Synnautes,* the two paralleling dorsal carinae extend posteriorly to a point approximately midway between the mandibles and the signature, ending abruptly. In typical *Halipeurus,* they continue on to roughly the level of the mandibles, where they again arc and join medially. On the ventral surface, a similar carina marks the ventromedial borders of the clypeus. It extends anteriorly to the level of the ante-clypeus. It may or may not be present in subgenus *Halipeurus,* but is very heavily sclerotized in *Synnautes.* At the posterior end, the postmarginal carina again arcs medially for a short distance. The marginal temporal carina tapers out caudad of the eye, becoming heavy again on either side of the posterior border of the gular sclerite. These last mentioned sclerotizations are sometimes referred to as 'occipital blotches.' The head of a third-instar nymph shows that the clypeus is divided medially prior to adulthood, and the dorsal carinae of adult *Halipeurus* demark this nymphal division.

The anterior plate, or signature, a separate sclerotized area lying between the premarginal carinae, is bordered by a broad hyaline margin anteriorly. Apparently movable, it is attached to the surrounding portions of the head with connective tissue. The exact function is unknown, but it is probably largely sensory. The ventral anterior border of the sclerotized portion is marked with scale-like undulations. Ventrally, just anterior of the mandibles, a soft, flap-like projection, the pulvinus, extends about half-way to the signature. The labium and the knob-like rudiments of the maxillae occupy the area immediately posterior of the mandibles. The rounded gular sclerite is situated between the mandibles and the posterior margin of the head.

The antennae are dimorphic (fig. 1). In typical *Halipeurus* males, the basal segment is approximately twice as long as the second, and varies, depending on the species, from cylindric to bulbous. The third segment is oblique distally, angling the last two segments dorsally and posteriorly, a condition that presumably aids the male in grasping the female during copulation. In the female, the antennae are simple, with the second segment as long or slightly longer than the first. Typical

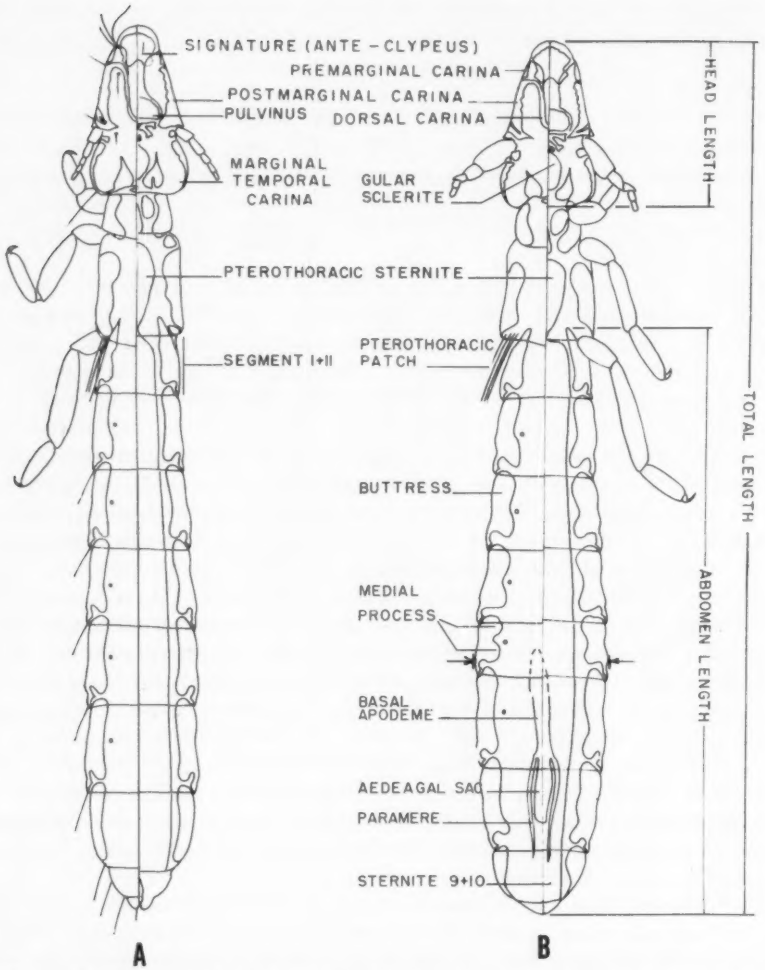

FIGURE 1. Diagrammatic drawings of typical *Halipeurus* to illustrate the principal terms and measurements. A, dorso-ventral view, female; B, dorso-ventral view, male.

Synnautes males have antennae that vary in much the same manner, but tend to be longer and slimmer.

The chaetotaxy of the head is relatively constant and offers little of taxonomic value. The setae inserted on the signature and the premarginal carina curve gracefully in front of the head and presumably serve as tactile organs.

Thorax: The thorax is typical, consisting of a distinct, relatively narrow, short prothorax, and a united meso- and metathorax, jointly termed the pterothorax. It is about as long as the head in this genus. As in the head, fusion and deletion have simplified the makeup of the thorax, but have also in turn obscured exact homologies.

With the exception of a patch of linearly arranged, close-set setae on the posterior border laterally, nothing is present on the thorax that is significant to this study. The setae mentioned, however, herein referred to as the pterothoracic patch, have some taxonomic interest and are mentioned in the descriptions. In the genus *Halipeurus,* there are usually three of these long setae set in a small unsclerotized area, with a small, stout seta adjoining laterally.

Abdomen: The abdomen is almost twice as long as the head plus the thorax. In the adults, there are nine apparent segments, since morphological segments 1 and 2 and 9 and 10 are fused. There is ample evidence in the nymphal stages that 9 and 10 are so fused in the adult, although none that 1 and 2 are (fig. 2, D and E). In the following discussions, the segments are referred to by their morphological

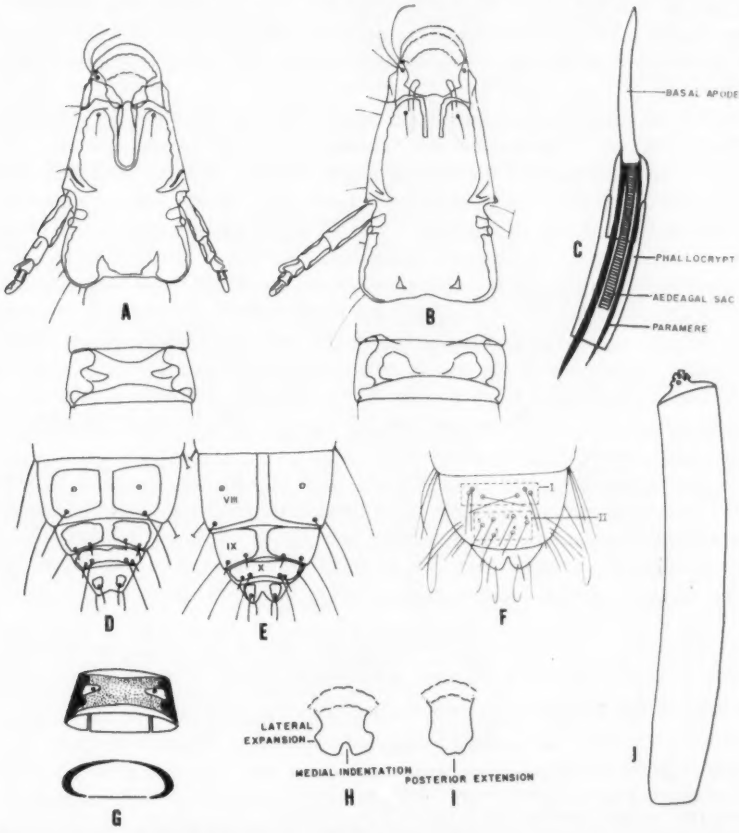

FIGURE 2. A, head and fifth abdominal segment of *H.* (*Halipeurus*) *taxosetus* sp. nov.; B, head and fifth abdominal segment of *H.* (*Synnautes*) *pelagicus* (Denny); C, diagrammatic drawing of male genitalia; D, terminal segment tergites of second-instar nymph; E, terminal segment tergites of third-instar nymph; F, schematic presentation of ventral chaetotaxy of terminal segments of adult male; G, diagrammatic views of abdominal sclerites; H, signature of *'taxosetus'* type, typical of lice from *Puffinus;* I, signature of *'procellariae'* type, typical of lice from *Pterodroma;* J, egg of *H. marquesanus* (Ferris) (cap is distorted).

number, i. e., apparent segment 5 is referred to as segment 6. With the exception of segment 11, each segment is strongly buttressed. These buttresses are relatively narrow in typical *Synnautes* and lack any marked development of medial processes (fig. 2, B). In *Halipeurus*, the buttresses are relatively wide and almost always have medial processes, especially in the middle segments, that involve much of the buttress. A diagrammatic view demonstrating the nature of the abdominal buttresses may be seen in fig. 2, G. The sixth segment is narrow in many species of this genus, allowing the abdomen to flex dorsally at a sharp angle to aid in copulation. This sixth segment retains, more obviously than the rest, the typical sclerotization of tergites of the *Philoceanus*-complex.

The terminal segments 9, 10, and 11 are of considerable interest, since the modifications of sclerites within this area enable one to separate out many of the species quickly. Males are usually more modified than are the females, and for this reason only males are designated as holotypes, a practice that I hope other workers will follow.

There is no doubt that, as mentioned previously, tergites 9 and 10 are fused in the adult, although it is questionable whether a similar condition exists in the sternites. The chaetotaxy of the sternite suggests that it is a result of such fusion, however, and it is considered to be so fused here. For the sake of convenience, the chaetotaxy is divided into two groups, I and II, as indicated in fig. 2, F. Group I remains fairly constant throughout the genus. Group II is often found in the condition figured, but, as the terminal segments are modified, these setae are shifted into other arrangements, and this shifting can be interpreted accurately in most cases.

The fact that the female terminal segment has buttresses suggests that the sclerotized remnants seen in segment 11 of the males are probably reduced buttresses. Typical female *Halipeurus* have a more or less truncate posterior with a rounded medial indentation, while *Synnautes* females have a bifid, pointed posterior, with enlarged setae terminating the buttresses.

The male genitalia consists basically of a pair of variously modified parameres attached proximally to a flat, lightly sclerotized basal apodeme. An aedeagal sac lies between the parameres and is usually only lightly, if at all, sclerotized. The parameres, plus the aedeagal sac, are retracted within an internal, tubular phallocrypt. In some species, at least, there appears to be an endophallus. (Refer to fig. 2, C for terminology.)

The egg is very long and slender (fig. 2, J), with a flat side. Anteriorly, it is obliquely truncate, bearing a lid with an irregular circle of about 15 micropyles, depending on the species.

Genus Halipeurus *Thompson*

Pediculus Linne, *partim*, 1758, Systema Naturae, 10th ed.
Lipeurus Nitzsch, *partim*, 1818, Germar's Magazin, **3**, p. 292.
Esthiopterum Harrison, *partim*, 1916, Parasit., **9**, pp. 21, 26.
Halipeurus Thompson, 1936, Ann. Mag. Nat. Hist., ser. 10, **18**, pp. 40–41. Genotype *Lipeurus angusticeps* Piaget.
Synnautes Thompson, 1936, As above, p. 43.
Halipeurus Harrison, 1937, Australasian Antartic Exped., 1911–14, ser. C, **2**, Pt. 1, p. 31. Genotype *Halipeurus euryphallis*, a nomen nudum.
Naubates Bedford, *partim*, T. Clay, 1940, Brit. Graham Land Exped., 1934–37, **1**, No. 5, pp. 309–310.

Description: Elongate lice adapted to living on the back and proximal parts of wings of *Puffininae* (shearwaters) and *Hydrobatidae* (storm petrels). Signature squarish, well devel-

oped. Head much longer than broad, with well developed dorsal carina extending posteriorly at least to level of pulvinus. Ventral carina indistinct to well developed. Antennae usually markedly dimorphic. Head and thorax about equal in length and breadth. Abdomen about twice as long as head plus thorax, straight-sided to spatulate, always widest in region of segment 7. Abdominal segments 9 and 10 almost indistinguishably fused. Sternite 9 and 10 without unusual sclerotizations or chaetotaxy, never divided medially, as in related genera. Genitalia with two variously modified parameres. Male genital opening ventral, sub-terminal.

Genotype: *Lipeurus angusticeps* Piaget.

Key to the Subgenera of Halipeurus

Dorsal carinae extending to, or almost to level of mandibles, then arcing medially and meeting in mid-line. Female abdomen rounded with slight, rounded medial indentation. Mid-abdominal segments of male with broad buttresses bearing well developed, rounded medial processes; tergites with heavily sclerotized, medially narrowed bands ..... Subgenus *Halipeurus*

Dorsal carina extend only to level of pulvinus and end abruptly. Female abdomen distinctly bifid, with deep, triangular indentation, buttresses of segment 11 each terminated with short, very broad seta. Mid-abdominal segments of male with narrow buttresses bearing poorly developed medial processes, usually only at posterior end of buttress. Tergites of male more evenly sclerotized than in *Halipeurus*. Cuticle with obvious scaly appearance. ..... Subgenus *Synnautes*

This key is detailed to avoid the equivocation present in the literature today. The two groups are almost generically distinct and easily recognized by anyone familiar with them. The overlap in some characters makes it presently undesirable to maintain these taxonts as separate genera, although additional material may prove that they should be so separated.

Subgenus Halipeurus *Thompson*

Halipeurus Thompson, 1936, Ann. Mag. Nat. Hist. ser. 10, **18,** pp. 40–41. Genotype *Lipeurus angusticeps* Piaget.

Description: Pre- and postmarginal carinae separate to almost joined. Premarginal carina tapered, not obliquely cut distally and interrupting outline of head. Dorsal carinae heavily sclerotized, extending to level of, or almost to mandibles, where they arc medially and meet in mid-line. Buttresses of abdomen relatively broad, those of middle segments particularly with well developed, rounded medial processes. Tergites in male with medially narrowed bands. Eleventh segment of female more or less rounded, with rounded medial indentation, without short stout setae terminating abdomen as in *Synnautes*.

Discussion: Twenty-two species and subspecies of the subgenus are described herein, of which 14 are new. In view of the fact that Kellogg saw many of these new species, and others that were new at the time, and referred them all to *diversus* Kellogg, a complete synonymy would be impossible. Even if Kellogg's host records were used as a basis, such a listing would be completely erroneous inasmuch as he or the people that collected for him were not careful of straggling, as evidenced by his collection. It would be hopeless to properly assign various species unless the actual specimens that he was referring to were at hand. The few that I have, kindly loaned by Dr. Ferris, are mostly stragglers, if the hosts listed on the slides are accurate.

The 22 species and subspecies recognized here may be divided into four major groups of species named as follows: *angusticeps, taxosetus, procellariae,* and *marquesanus* species groups. The first, *angusticeps,* may be readily distinguished by the asymmetry of the terminal segments. It includes three species and one subspecies. There is no way to determine from which of the other groups it evolved, although it seems to be *taxosetus.* In any event, there is no doubt that it is a derivative group. The second, *taxosetus,* is distinguished principally by the shape of the signature. Of the 11 species included within it, 9 are typically parasitic on the birds of the genus *Puffinus.* The third, *procellariae* (three species), and fourth, *marquesanus* (three species), are distinguished from *taxosetus* principally on the basis of the shape of the signature. The relative length of male segment six, near the length

of the fifth in *marquesanus* and only about one-half the length of the fifth in *procellariae*, readily separates these two groups. Both *procellariae* and *marquesanus* species groups are typically parasitic on birds of the genus *Pterodroma*.

Key to the Species Groups and Species of Subgenus Halipeurus

This key is primarily based on males, except where females are specifically mentioned. The females by themselves are very difficult to determine, the best technique being to compare them critically with previously determined females of the same size range. When familiar with the lice, one may quickly place females by virtue of certain qualitative aspects that cannot be successfully applied in a key.

1. With obvious asymmetry of part or all of sclerites of terminal segments ..... *angusticeps* species group
   Without asymmetrical developments of sclerites of terminal segments 2
2. Signature with lateral expansions and/or without narrow posterior extension ..... *taxosetus* species group
   Signature with greatly reduced or no lateral expansions, and with narrow posterior extension 3
3. Female terminal segments typical, not apparently fused into a single, evenly sclerotized unit, medial indentation obvious ..... *procellariae* species group
   Female terminal segments apparently fused into a single, more-or-less evenly sclerotized unit, medial indentation greatly reduced or absent; posterior border of segment 11 broad and squared; males without obvicusly shortened abdominal segment ..... *marquesanus* species group

angusticeps *species group*

1. Sternite 9 and 10 only asymmetrical ..... *angusticeps* subspp. 2
   Segment 11 markedly asymmetrical 3
2. Sternite 9 and 10 with irregular posterior margin, sharply angled caudad to right; total length greater than 3.6 mm. ..... *a. angusticeps*
   Sternite 9 and 10 as above, but less irregular and less sharply angled; total length less than 3.6 mm. ..... *a. fosteri*
3. Buttresses of 11th segment only involved in knob-like postanal extension on the right side ..... *mirabilis*
   Buttresses of segment 9 and 10 and 11 fused on right side, and form hook-like postanal extension ..... *abnormis*

taxosetus *species group*

1. Posterior border of sternite 9 and 10 triangular, heavily sclerotized, with completely atypical setal pattern 9
   Sternite 9 and 10 without excessive modification 2
2. Parameres short, broadened, and widely set apart 10
   Parameres slender, not widely set apart. 3
3. With a row of three closely set setae on each latero-posterior border 7
   Without such a row 4
4. Parameres slender, equal or only slightly sub-equal in length. 5
5. Parameres subspatulate, relatively broad, and differing greatly in length; group II setae somewhat irregular in pattern. ..... *placodus*
   Parameres very slender, not varying in width 6
   Parameres slender, but variable in width, from specimen to specimen and point to point along each paramere ..... *diversus*
6. Sternite 9 and 10 with rounded, heavily sclerotized corners, presenting superficial 'pincers-like' appearance. ..... *forficulatus*
   Sternite not so modified, group 11 setae distinctly caudad on sternite. ..... *bulweriae*
7. More than 3.0 mm in length, parasites of *Puffinus*. ..... *taxosetus*
   Less than 3.0 mm in length, parasites of *Pelecanoides* ..... *falsus* subspp. 8
8. Signature more-or-less *diversus*-type, but irregular. ..... *f. falsus*
   Signature distinctive, with large, regularly expanded lateral expansions, narrowing smoothly to small but distinctive medial indentation. ..... *f. pacificus*

9. Parameres slender, lightly sclerotized; 11th segment heavily sclerotized but not greatly reduced ..... *thompsoni*
Parameres not so slender, heavily sclerotized; aedeagal sac with curious sclerotizations; a large species with 11th segment greatly reduced ..... *micariproctus*
10. Sternite 9 and 10 with longitudinally drawn out setal pattern; signature very long, rectangular in shape. ..... *attenuatus*
Sternite 9 and 10 with fairly typical setal pattern 11
11. Basal segment of antenna almost bulbous; with slightly irregular setal pattern; aedeagal sac almost as long as parameres; over 3.5 mm in length ..... *mundae*
Basal segment of antenna sub-bulbous; with group II setae 5 and 6 distinctly laterad; aedeagal sac with rod-like, obliquely cut sclerotization, less than 3.5 mm in length. ... *turtur*

procellariae *species group*

1. Parameres relatively broad, of medium length, with spatulate tips, subequal and only slightly asymmetrical; with variously modified rod-like sclerotizations within aedeagal sac. 2
Parameres equal in length, symmetrically curved mediad; without sclerotization within aedeagal sac. ..... *procellariae*
2. Sternite 9 and 10 rectangular, with typical setal pattern ..... *kermadecense*
Sternite 9 and 10 with broadly rounded posterior margin. ..... *accentor*

marquesanus *species group*

1. Parameres long and slender 2
Parameres short and relatively broad. ..... *intermedius*
2. Sternite 9 and 10 with narrow, rectangular extension overlapping 11th segment. *marquesanus*
Sternite 9 and 10 without such extension, group II setae crowded posteriorly. *postmarquesanus*

Halipeurus (Halipeurus) angusticeps (Piaget)

Lipeurus angusticeps Piaget, 1880, Les Pediculines, pp. 306–308, pt. 25, fig. 4. *Type-host: Procellaria cinerea.*

Description: A medium-sized species, ± 3.5 mm. Characterized by the irregular, obliquely cut posterior border of sternite 9 and 10 in the male. Signature with deep, triangular medial indentation in female. See descriptions of subspecies for detailed information.

Halipeurus (Halipeurus) angusticeps angusticeps (Piaget)
(Figs. 3–7, A)

Lipeurus angusticeps Piaget, 1880, Les Pediculines, pp. 306–308, pl. 25, fig. 4. *Type-host: Procellaria cinerea.*
Esthiopterum angusticeps (Piaget). Harrison, 1916, Parasit., **9**, No. 1, p. 130.
Halipeurus angusticeps (Piaget). Thompson, 1936, Ann. Mag. Nat. Hist., ser. 10, **18**, p. 41. Designated as genotype.

Description: Head ratio 2.2. Signature with rounded medial indentation, pointed in female. Basal segment of antenna sub-bulbous. Dorsal carina extends to mandibles, only lightly sclerotized in male. Abdomen ratio 5.8, narrow. Sixth segment as long as fifth. Segments 4, 5 and 6 with well developed paratergal processes, 7, 8 and 9 and 10 without processes. Posterior border of sternite 9 and 10 asymmetrical, cut obliquely downward to right and distinctly irregular. Terminal segments relatively attenuate. Eleventh segment bilobed, relatively unsclerotized, deeply indented. Parameres slender, lightly sclerotized, right paramere shorter and arced medially. Basal apodeme indistinct. Female typical.

Type-host: Unknown. Listed as *Procellaria cinerea* by Piaget, presumably meaning *Adamastor cinereus* (Gmelin).

Measurements: A single male and female, in millimeters:

	Male	*Female*
Total length:	3.84	4.63
Length of head:	0.85	
Width of head:	0.39	
Length of abdomen:	2.38	
Width of abdomen:	0.41	

Material examined: one male, one female, from *Procellaria cinerea* from the Piaget Collection, slide no. 445, BMNH no. 1928–325, kindly loaned by Miss Clay. Male on above slide designated as lectotype. Two male and three female paratypes from same material, all specimens in the British Museum (Natural History).

Discussion: Careful examination of almost 75 museum skins of *Adamastor cinereus* (Gmelin) failed to uncover any specimens of this louse. This seems strange, since the lice of this subgenus are usually reasonably easy to obtain from

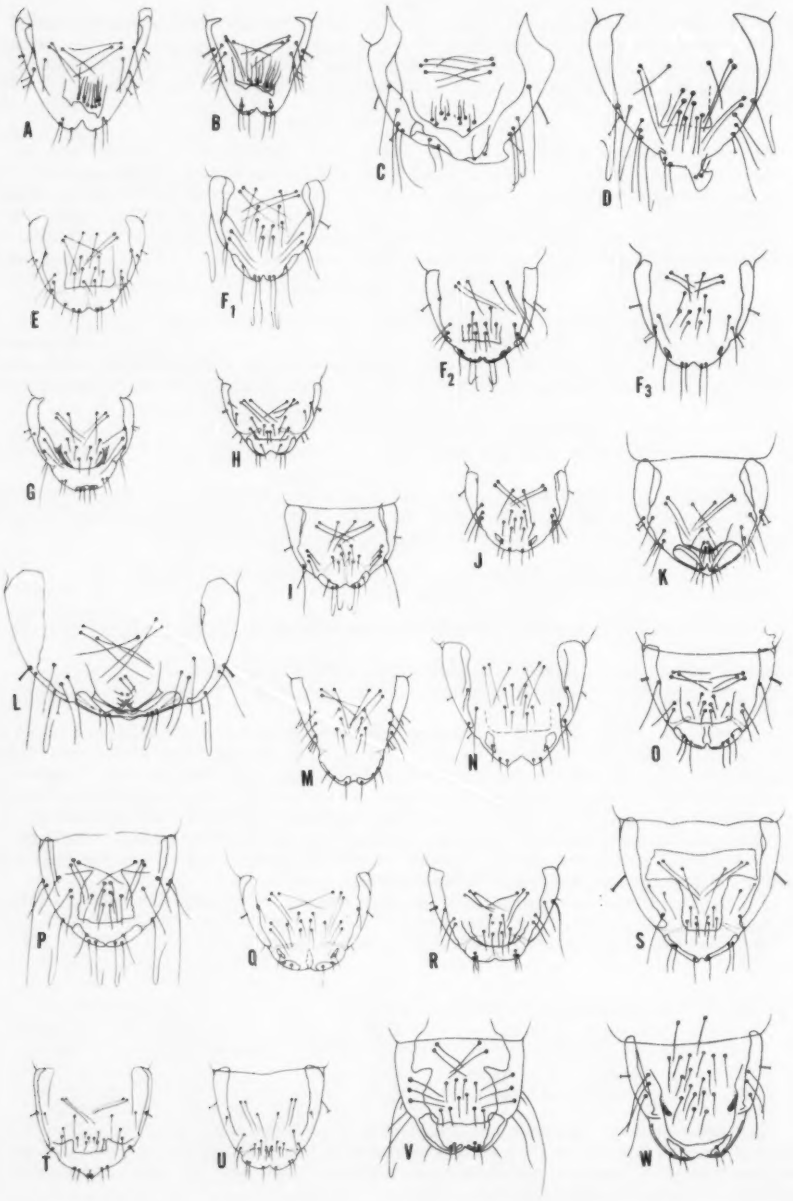

FIGURE 3. Ventral chaetotaxy of male terminal segments.

The diagnostic drawings of figures 3, 4, 5, 6, and 7 are consistently lettered (as listed below) to facilitate their use. They represent specimens from the type-host unless otherwise noted.

A. *Halipeurus* (*Halipeurus*) *a. angusticeps* (Piaget)
B. *Halipeurus* (*Halipeurus*) *a. fosteri* subsp. nov.
C. *Halipeurus* (*Halipeurus*) *abnormis* (Piaget)
D. *Halipeurus* (*Halipeurus*) *mirabilis* Thompson
E. *Halipeurus* (*Halipeurus*) *placodus* sp nov.
F. *Halipeurus* (*Halipeurus*) *diversus* (Kellogg)
   1, specimens from type host *Puffinus griseus* (Gmelin)
   2, specimens from *Puffinus l. boydi* Mathews
   3, specimens from *Puffinus p. yelkouan* (Acerbi)
G. *Halipeurus* (*Halipeurus*) *forficulatus* sp. nov.
H. *Halipeurus* (*Halipeurus*) *bulweriae* sp. nov.
I. *Halipeurus* (*Halipeurus*) *taxosetus* sp. nov.
J. *Halipeurus* (*Halipeurus*) *falsus* Eichler
   1, specimens from *Pelecanoides garnotti* (Lesson), the type host
   2, specimens from *Pelecanoides urinatrix* (Gmelin), type host of *H. f. pacificus* subsp. nov.
K. *Halipeurus* (*Halipeurus*) *thompsoni* sp. nov.
L. *Halipeurus* (*Halipeurus*) *micariproctus* sp. nov.
M. *Halipeurus* (*Halipeurus*) *attenuatus* sp. nov.
N. *Halipeurus* (*Halipeurus*) *mundae* sp. nov.
O. *Halipeurus* (*Halipeurus*) *turtur* sp. nov.
P. *Halipeurus* (*Halipeurus*) *procellariae* (Fabricius)
Q. *Halipeurus* (*Halipeurus*) *kermadecense* (Johnston and Harrison)
R. *Halipeurus* (*Halipeurus*) *accentor* sp. nov.
S. *Halipeurus* (*Halipeurus*) *intermedius* nom. nov.
T. *Halipeurus* (*Halipeurus*) *marquesanus* (Ferris)
U. *Halipeurus* (*Halipeurus*) *postmarquesanus* sp. nov.
V. *Halipeurus* (*Synnautes*) *pelagicus* (Denny)
W. *Halipeurus* (*Synnautes*) *ncsofregettae* sp. nov.

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skins. None of the other species of birds of the subfamily Puffininae examined carried specimens of *angusticeps*.

This species of louse is very closely related to that parasitizing *Puffinus leucomelas*, and, in view of the opinion that *leucomelas* is closely, perhaps subspecifically related to *Puffinus kuhlii*, *H. a. angusticeps* may parasitize a local population of *kuhlii*. However, all of the skins of the various subspecies of *kuhlii* examined carried specimens of *H. abnormis* (Piaget) only.

Halipeurus (*Halipeurus*) *a. fosteri* subsp. nov.
(Figs. 3–7, B)

Description: Smaller, but essentially as *H. a. angusticeps*, differing as follows: Head ratio 2.1. Dorsal carina typical, except only lightly sclerotized near level of mandibles. Abdomen ratio 6.0. Sternite 9 and 10 with irregular posterior margin, obliquely cut to right, heavily sclerotized, but not sloped as sharply and less irregular. Right paramere only slightly curved medially.

Measurements: 9 males, 5 females, averages and ranges, in millimeters:

	Males	*Females*
Total length	3.53 (3.38 to 3.54)	4.38 (4.31 to 4.46)
Length of head	0.75 (0.73 to 0.78)	
Width of head	0.36 (0.36 to 0.37)	
Length of abdomen	2.24 (2.16 to 2.32)	
Width of abdomen	0.37 (0.34 to 0.41)	

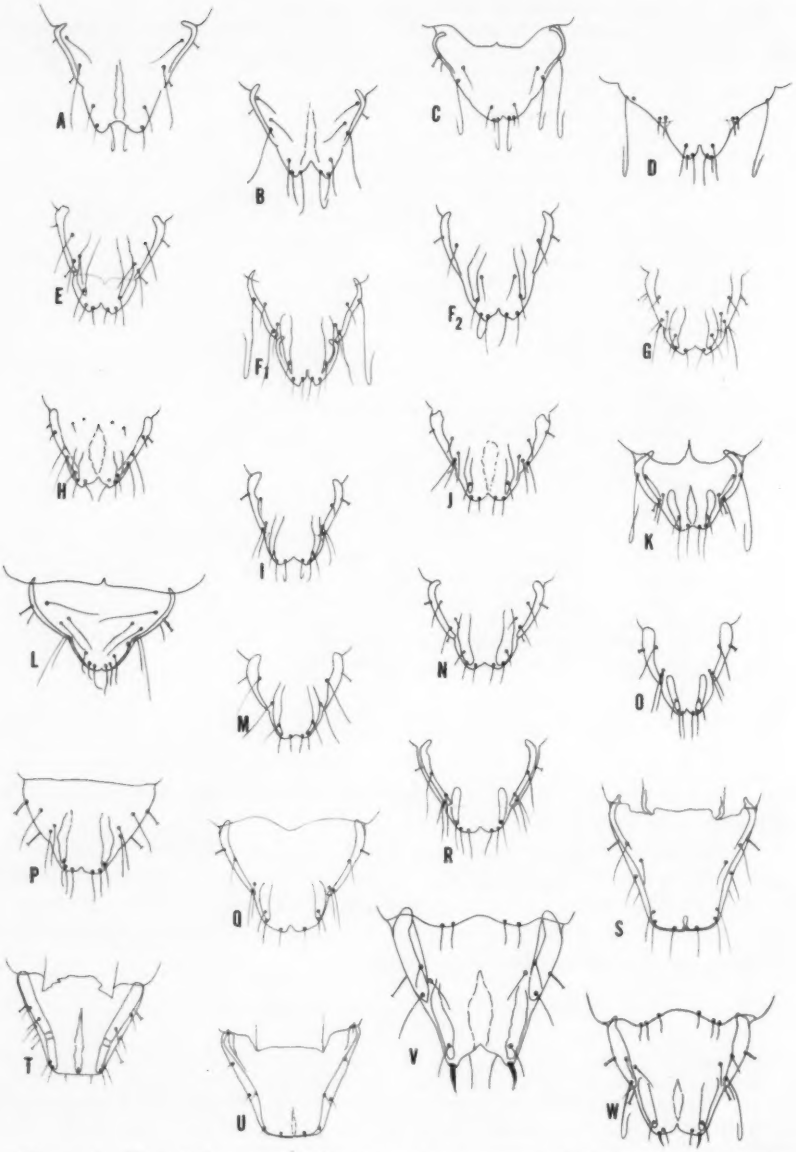

FIGURE 4. Ventral chaetotaxy of female terminal segments. (Labeling same as for figure 3.)

Type-host: Puffinus leucomelas (Temminck).

Material Examined: All from the type-host as follows: Holotype male, allotype female, 1 male and 8 female paratypes, from MCZ skin no. 131515, collected from China Sea between Shanghai and Foochow (RLE); 1 male, 1 female, paratypes, from 3° 10′ S, 155° E, no further data (AMNH) slide); 4 male, 6 female paratypes, from New Guinea, no further data (GBT); 3 male, 6 female paratypes from AMNH skin no. 220603, collected at 3° 10′ S, 155° E (RLE).

Halipeurus (Halipeurus) abnormis (Piaget)
(Figs. 3–7, C)

Lipeurus abnormis Piaget, 1885, Les Pediculines, sup., pp. 65–66, pl. 7, fig. 2. *Type-host: Puffinus major.*

Esthiopterum abnorme (Piaget). Harrison, 1916, Parasit., **9**, No. 1, p. 129.

Halipeurus abnormis (Piaget). Thompson, 1938, Ann. Mag. Nat. Hist., ser, 11, **2**, p. 482.

Description: A large species, head ratio 1.8, signature squarish; rounded posterior indentation, usually shallow and indistinct. Basal segment of antenna bulbous, second enlarged, distal two segments relatively small; first three segments more or less coalesced into a single tapered unit. Abdomen ratio 4.8, relatively broad, spatulate. Paratergites as in *H. angusticeps* and *fosteri*. Segment 6 about three-fourths as long as 5th. Terminal segments assymmetrical, heavily sclerotized. Paratergites 9, 10, and 11 fused and terminally arced laterad on right, 11th separate on left. Sternite 9 and 10 broad, heavily sclerotized and slightly asymmetrical, pattern typical, slightly distorted. Genitalia also heavily sclerotized, with parameres broadened out basically as in *H. a. fosteri*. Many irregular sclerotizations within or upon aedeagal sac. Female with distinct ventral clypeal bands. Terminal segments short, weakly sclerotized.

Measurements: 7 males, 10 females, averages and ranges in millimeters, from *Puffinus kuhlii edwardsi:*

	Males	*Females*
Total length	4.36 (4.15 to 4.54)	4.97 (4.76 to 5.38)
Length of head	0.89 (0.85 to 0.92)	
Width of head	0.49 (0.46 to 0.51)	
Length of abdomen	2.77 (2.62 to 2.93)	
Width of abdomen	0.58 (0.54 to 0.65)	

Type-host: Puffinus gravis (O'Reilly)

Material Examined: From various subspecies of *Kuhlii* and the type-host as follows:

Puffinus k. kuhlii (Scopoli): 1 male, 1 female, from MCZ skin no. 47230, South Spain (RLE): 3 males, 8 females, from Greece and Spain, (GBT).

Puffinus k. borealis Cory: 8 males, 16 females, from MCZ skin no. 135290, Azores, (RLE).

Puffinus k. edwardsii (Oustalet): 7 males, 16 females from MCZ skin no. 94293, (RLE).

Puffinus k. flavirostris (Gould): 1 male, Cape Seas, (GBT).

Puffinus gravis (O'Reilly): Male (here designated as lectotype) in the Piaget Collection, BMNH slide no. 454. Four male and two female paratypes.

Discussion: This large species is obviously related to *H. a. fosteri* and *H. angusticeps*. It differs, however, in that it has an unusual asymmetrical development of its terminal segment. The parameres are basically the same shape as in the above mentioned species, but are broader, more heavily sclerotized, and overshadowed by the sclerotizations in the aedeagal sac.

Puffinus gravis carries two species of *Halipeurus; abnormis* and *micariproctus* sp. nov. It is unusual for a host to be parasitized by two so closely related species. There seems little doubt, however, that this situation is a true one. It would be interesting to know if any one individual bird has thriving populations of both of these *Halipeurus* species.

Halipeurus (Halipeurus) mirabilis Thompson
(Figs. 3–7, D)

Halipeurus mirabilis Thompson, 1940, Ann. Mag. Nat. Hist., ser. 11, **5**, pp. 499–502, text figs. 1–5, pl. 10, figs. 1, 2.

Type-host: Puffinus pacificus chlororhynchus Lesson.

Description: Head ratio 2.0. Signature with broadly rounded posterior indentation. Basal

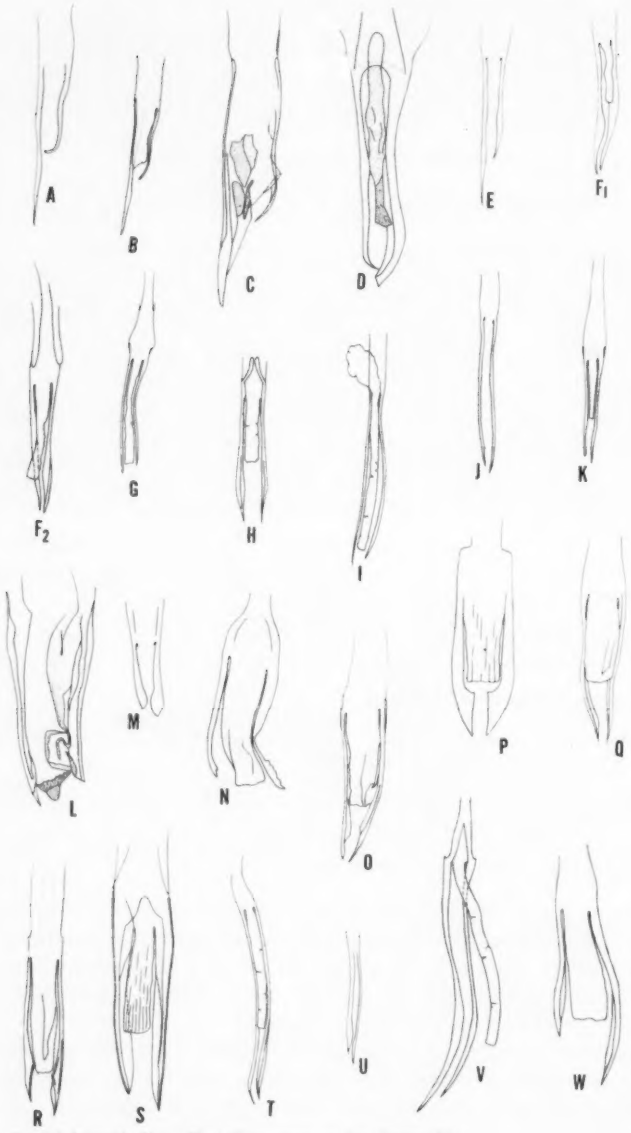

FIGURE 5. Male genitalia. (Labeling same as for figure 3.)

segment of antenna bulbous, second segment only slightly enlarged. Abdomen ratio 5.1, subspatulate. Sixth segment slightly longer than fifth. Paratergites as in *H. angusticeps*. Eleventh segment has heavily sclerotized asymmetrical developments, with knob-like extension on right side. Sternite 9 and 10 narrow, sclerotized, with typical but slightly distorted setal pattern. Genitalia heavily scrclotized, subequal, parameres broad, curved mediad distally.

Measurements: 5 males and 6 females, averages and ranges in millimeters:

	Males	*Females*
Total length	4.50 (4.38 to 4.62)	4.64 (4.62 to 4.70)
Length of head	0.83 (0.82 to 0.85)	
Width of head	0.43 (0.41 to 0.44)	
Length of abdomen	3.04 (2.92 to 3.08)	
Width of abdomen	0.59 (0.58 to 0.64)	

Type-host: Puffinus pacificus chlororhynchus Lesson.

Material examined: 1 male, 2 female paratypes from the type-host, kindly loaned by Mr. G. B. Thompson, the original describer. Other material as follows:

Puffinus pacificus chlororhynchus Lesson: 4 males, 4 females, from AMNH skin collected near Banks Island (RLE).

Puffinus pacificus pacificus (Gmelin): 4 males, 8 females, collected near Sunday, Kermadec Islands (RLE).

Puffinus pacificus cuneatus Salvin: 2 males, 5 females from MCZ skin no. 239641, Oahu, Hawaiian Islands (RLE).

Discussion: The relationship of other species to this one is obscure, although it seems to be related to those previously discussed. It is easily distinguished by the asymmetrical developments of the 11th segment.

Halipeurus (Halipeurus) placodus sp. nov.
(Figs. 3-7, E)

Description: Like *diversus*, from many hosts and variable. Head ratio 2.1. Signature with shallow medial indentation. Abdomen ratio 5.8, broader relatively than *diversus*, slightly broadened posteriorly. Buttresses with well developed medial processes. Sixth segment less than one-half as long as fifth. Terminal segments similar to *diversus* with less sclerotization of 11th and considerably more of sternite 9 and 10. Setal pattern of sternite 9 and 10 typical, with almost no variation. Parameres of genitalia distinctly subequal, broadened distally with pointed tips. Basal apodeme broad, moderately sclerotized.

Type-host: Puffinus assimilis tunneyi Mathews.

Measurements: Averages and ranges in millimeters of 6 males, 6 females, from type-host:

	Males	*Females*
Total length	3.37 (3.31 to 3.39)	4.08 (4.00 to 4.15)
Length of head	0.69 (0.68 to 0.72)	
Width of head	0.33 (0.32 to 0.34)	
Length of abdomen	2.05 (2.00 to 2.16)	
Width of abdomen	0.35 (0.34 to 0.37)	

Material examined: From the type-host; holotype male, allotype female, 3 male, 5 female paratypes, from MCZ skin no. 166366, West Wallaby Island, Australia, (RLE). From other hosts as follows:

Puffinus assimilis assimilis Gould: 4 males, 7 females from MCZ skin no. 236975, collected near the Herald Islands, South Pacific, (RLE).

Puffinus assimilis kermadecensis Murphy: 10 male, 11 female paratypes collected near Kermadec Islands, (RLE).

Puffinus l'herminieri nugax Mathews: 2 male, 4 female paratypes, from AMNH skin no. 216890, collected near New Hebrides, (RLE).

Puffinus auricularis Townsend: 3 males, stragglers?, MCZ skin from Charion Islands, (RLE).

Discussion: The apparently anomalous distribution of this louse on various host species illustrates the ever-present danger of leaning too heavily on parasitic distribution as a clue to host relationship. This is especially true at the species level of the louse. This species, *placodus*, may be found on three subspecies of *Puffinus assimilis* as well as one subspecies of *P. l'herminieri* and perhaps lends some slight support to Murphy's (1927) division of *assimilis* into two species, *assimilis* and *l'herminieri*.

This species can be readily separated from *diversus* which it superficially resembles by the unequal parameres, the typical setal pattern, the shorter 6th abdominal segment, the more obviously sclerotized sternite 9 and 10 with its distinct posterior margin, and the narrower abdomen. The females may be separated by size differences only.

Halipeurus (*Halipeurus*) *diversus* (Kellogg)
(Figs. 3–7, F)

Lipeurus diversus Kellogg, 1896, New Mallophaga 1, pp. 123–124, pl. 8, figs. 3, 4. *Type-host: Puffinus opisthomelas* Coues.
Lipeurus limitatus Kellogg, as above, pl. 8, figs. 5, 6. Immature of *diversus*. *Type-host: Puffinus griseus* (Gmelin).
Esthiopterum diversum (Kellogg). Harrison, 1916, Parasit., **9**, No. 1, p. 133.
Esthiopterum constrictiventre Pessoa and Guimaraes, 1935, Ann. Fac. Med., Sao Paulo, fasc. 3, **11**, pp. 5–6, figs. 6, 7. *Type-host: Aestrelata macroptera* Smith.
Halipeurus diversus (Kellogg). Thompson, 1938, Ann. Mag. Nat. Hist., ser. 11, **2**, p. 485.

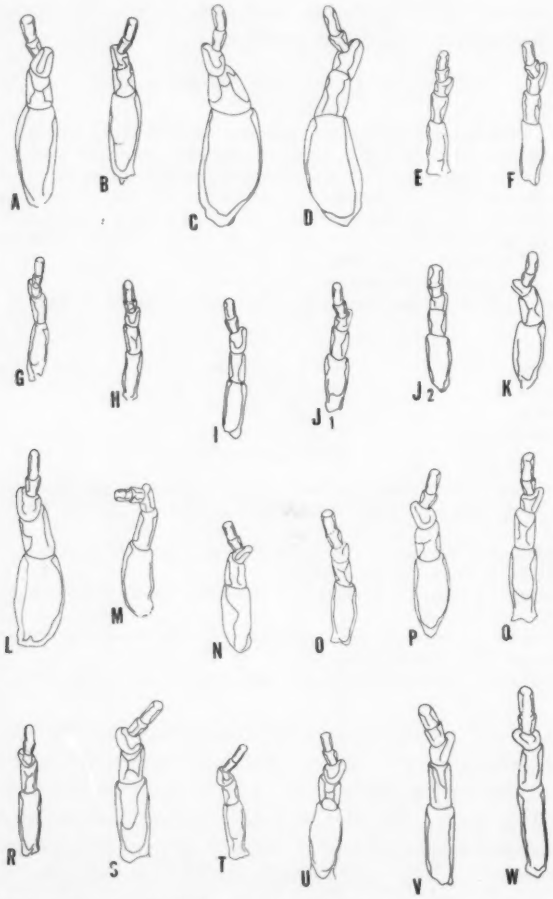

FIGURE 6. Male antennae. (Labeling sames as for figure 3.)

Description: Head ratio 2.0. Signature with well-developed lateral extensions and shallow medial indentation. Basal segment cylindric. Abdomen ratio 6.1, almost straight-sided. Sixth segment one-half or more longer than 5th. All paratergal buttresses with medial processes. Paratergite 9 and 10 relatively long, slender, tapering. Setal pattern of sternite 9 and 10 almost typical, except that setae nos. 1 and (especially) 2 vary in position anteriorly. Seta no. 2 usually displaced further anteriorly, often paralleled by nos. 5 and 6, (specimens from *Puffinus puffinus* subspp. vary the most). Sternite 9 and 10 sufficiently sclerotized so that posterior margin is especially distinct. Parameres long, relatively broad, barely subequal.

Measurements: 10 males and 10 females from *Puffinus griseus,* averages and ranges as follows:

	Males	*Females*
Total length	3.69 (3.53 to 3.84)	4.36 (4.15 to 4.53)
Length of head	0.76 (0.73 to 0.78)	
Width of head	0.38 (0.36 to 0.39)	
Length of abdomen	2.27 (2.15 to 2.31)	
Width of abdomen	0.37 (0.34 to 0.39)	

Type-host: Puffinus griseus (Gmelin), see discussion.

Material examined: Kellogg types, 2 males, 1 female, host labeled as *Puffinus opisthomelas,* Pacific Grove, Calif., slide no. 140b, and types of *Lipeurus limitatus,* similarly labeled, slide no. 140c, kindly loaned me by Dr. G. E. Ferris, Stanford University. Other material as follows:

Puffinus griseus (Gmelin): 13 males, 25 females, off Moki Hinau, New Zealand (RLE); 1 male, 1 female, Depoe Bay, Oregon, H. S. Fuller, collector.

Puffinus tenuirostris (Temminck): 11 males, 10 females, from MCZ skin no. 318119, collected near Kodiak Island, Alaska (RLE); 3 males, 1 female from Bass Straits, New Zealand (RLE).

Puffinus puffinus puffinus (Brunnich): 1 male, 4 females from Mar del Plata, Argentina, (RLE); 15 males, 13 females, from British Isles, (Meinertzhagen); 3 males, 7 females, from British Isles, (GBT).

Puffinus puffinus yelkouan (Acerbi): 4 females, from MCZ skin, collected at Majorca, (RLE).

Puffinus puffinus mauretanicus Lowe: 3 females, from MCZ skin, British Isles, (RLE); 1 female, Balearic Islands, (Meinertzhagen).

Puffinus l'herminieri boydi Mathews: 4 males, 10 females, from AMNH skin no. 527894, Robbos Group, Cape Verde Islands, (RLE); 1 male and 3 females, Cape Verde Island (Meinertzhagen).

Pterodroma macroptera macroptera Smith: 1 male, 1 female compared with type of *Esthiopterum constrictiventre,* collected at Guaruja, S. P., Brazil, kindly sent to me by Dr. Lindolfo Guimaraes. There are almost certainly stragglers, *Halipeurus procellariae* (Fab.) usually parasitizing this host.

Discussion: Kellogg's types of *diversus* are almost certainly stragglers or the result of a mix-up during processing. The type-host, *Puffinus griseus,* was chosen because it harbors this species of louse commonly and because Kellogg assigns the type-host of the nymph, which he described as *Lipeurus limitatus,* as *Puffinus griseus* in the text. This, despite the fact that the type label gives *Puffinus opisthomelas* as the host.

This species ranges almost around the world on its various hosts. Although differences can be seen in populations from each of these species, the range of differences in each precludes the possibility that they have subspeciated to the extent that each host bears lice that have a consistent morphological identity. The lice from *Puffinus l. boydi* (figs. 3 and 5, F-2) range from typical *diversus* to a condition in which sternite 9 and 10 is obviously more heavily sclerotized, while those from *Puffinus p. yelkouan* (figs. 3, 4 and 7, F-3) range from typical *diversus* to specimens having only a minute amount of sclerotization in the terminal segments.. This unusual amount of variation from collection to collection within each host species strongly suggests that changes are presently taking place.

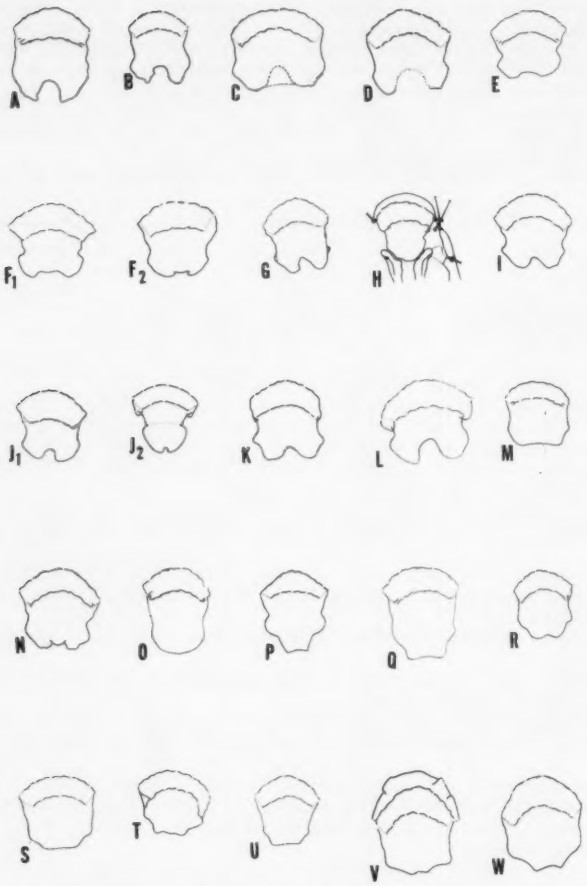

FIGURE 7. Male signatures. (Labeling same as for figure 3.)

Halipeurus (Halipeurus) forficulatus sp. nov.
(Figs. 3–7, G)

Description: Head ratio 2.3, very narrow. Signature elongated, relatively straight-sided; relatively deep, irregularly rounded medial indentation. Basal segment of antenna cylindric. Abdomen ratio 6.8, narrow and straight-sided. Segments 2 to 8 with well developed medial processes on buttresses. Sixth segment slightly less than one-half as long as 5th. Eleventh segment rounded, no medial division, with distinct apical sclerotizations. Sternite 9 and 10 heavily sclerotized, broadly rounded posteriorly, with lateral margins built up and irregularly scalloped, presenting the appearance of a pair of pincers. Parameres long and slender, barely subequal, with aedeagal sac almost as long as shortest paramere.

Measurements: 10 males, 10 females from all hosts, averages and ranges in millimeters:

	Males	*Females*
Total length	3.36 (3.15 to 3.54)	3.94 (3.85 to 4.00)
Length of head	0.69 (0.65 to 0.71)	

Width of head	0.30 (0.28 to 0.31)
Length of abdomen	2.11 (2.00 to 2.24)
Width of abdomen	0.31 (0.27 to 0.34)

Type-host: *Puffinus l'herminieri polynesiae* Murphy.

Material examined: From the type-host: Holotype male and allotype female, 4 male and 5 female paratypes, from MCZ skin no. 191742, collected near Manoui, (RLE); 3 male, 2 female paratypes, Marquesas Islands, (BMNH).

Puffinus l'herminieri dichrous Finsch and Hartlaub: 3 male, 5 female paratypes, from AMNH skin, collected near Palau, Caroline Islands, (RLE).

Puffinus heinrothi Reichenow: 3 males from AMNH skin no. 230280, New Britain, (RLE). Stragglers?

Discussion: A distinctive louse; the modification of sternite 9 and 10 and the elongate signature serve to separate it from all other species. If the record from *Puffinus heinrothi* is valid, the list of hosts again suggests that geography, with the aid of an unknown agent, sometimes has more to do with the distribution of these lice than the relationships of the hosts.

Halipeurus (*Halipeurus*) *bulweriae* sp. nov.
(Figs. 3–7, H)

Description: A small species, superficially like *Synnautes*. Head ratio 2.4, very narrow. Signature elongated, rounded posterially. With obvious hiatus between postmarginal carina and dorsal carina. Premarginal and postmarginal carinae are virtually fused. Abdomen ratio 6.8, narrow and straight-sided. Buttresses very narrow, with knob-like medial processes developed only on posterior ends. Segment 6 about one-half the length of 5. Parameres long, slender, only slightly sub-equal, curved mediad slightly at tips. Aedeagal sac almost as long as parameres.

Measurements: 5 males, 5 females from the type-host, averages and ranges in millimeters.

	Males	*Females*
Total length	3.30 (3.15 to 3.46)	3.78 (3.70 to 3.85)
Length of head	0.69 (0.67 to 0.72)	
Width of head	0.29 (0.27 to 0.31)	
Length of abdomen	2.13 (2.07 to 2.22)	
Width of abdomen	0.31	

Type-host: *Bulweria bulweria* (Jardine and Selby).

Material examined: Holotype male, allotype female, 2 males, 3 female paratypes, from type host, from MCZ skin collected near Canary Islands, (RLE); 1 male, 2 female paratypes, no data, (GBT); 1 male, 2 female paratypes, Canary and Madeira Islands, (Meinertzhagen).

Halipeurus (*Halipeurus*) *taxosetus* sp. nov.
(Figs. 3–7, I)

Description: Similar to *H. diversus*. Head ratio 2.0. Signature variable, usually slightly elongate compared with *H. diversus*, with pronounced lateral extensions and rounded medial indentation. Basal segment of antenna cylindric. Abdomen ratio 6.2. Buttresses with well developed medial processes. Segment 6 slightly more than one-half as long as 5. Eleventh segment with lightly sclerotized buttress, rounded, with only a slight medial indentation. Sternite 9 and 10 with typical setal pattern, two rows of three closely set, stouter setae laterad of group II. Parameres long and slender, slightly subequal. Female typical.

Measurements: 10 males and 10 females from *Puffinus nativitatis*, averages and ranges in millimeters.

	Males	*Females*
Total length	3.39 (3.31 to 3.54)	3.93 (3.85 to 4.00)
Length of head	0.70 (0.68 to 0.73)	
Width of head	0.34 (0.33 to 0.35)	
Length of abdomen	2.10 (2.00 to 2.16)	
Width of abdomen	0.34 (0.32 to 0.35)	

Type-host: *Puffinus nativitatis* Streets.

Material examined: From the type-host, holotype male, allotype female, 20 male, 27 female paratypes, from Dukie Island, and Christmas Island, collected by R. H. Beck (AMNH); 8 male, 12 female paratypes, from MCZ skins, Laysan Island (RLE).

Puffinus gavia (Forster) (=*P. reinholdi* Mathews): 8 males, 5 females, collected by R. H. Beck at 44 S, 173 W. (AMNH).

Puffinus opisthomelas Coues: 8 males, 8 females, from MCZ skin, Monterey and Santa Monica, Calif., (RLE).

Puffinus l'herminieri l'herminieri Lesson: 9 males, 14 females, from MCZ skin no. 90670, Ginger Island, Bahamas, (RLE), 1 male, 1 female, from the Bahamas, (GBT).

Two males, 2 females, Krusenstern Island, (GBT). Other material as follows:

Puffinus l'herminieri bannermani Mathews and Iredale: 2 males, 2 females, from MCZ skin no. 82908, near North Iwo Jima, Bonin Islands, (RLE).

Puffinus l'herminieri bailloni (Bonaparte): 1 male, from AMNH skin collected at Prustin Island, Seychelles, (RLE).

Puffinus assimilis baroli (Bonaparte): 2 males, 4 females, from MCZ skin no. 42011, collected near Porto Santo, Madeira Islands, (RLE).

Discussion: Closely related to *Halipeurus falsus* Eichler and possibly conspecific with it. I believe that these two species should be left as is, pending further material from *Pelecanoides* and knowledge of these lice in general. It differs in the shape of the parameres, size generally and in length of the parameres. The usually conservative females can be separated instantly on the basis of the shape of the signature. With the above noted exception, it can be instantly separated from all other species on the basis of the two rows of three closely set setae on sternite 9 and 10. The sclerotization of the 11th segment links this species with *Halipeurus thompsoni.*

Halipeurus (Halipeurus) falsus Eichler

Halipeurus falsus Eichler, 1949, Rev. Brasil. Biol. **9**, No. 3, pp. 338, text figs. 2, 3. Type-host: *Pelecanoides garnotti* (Lesson).

Description: Essentially as *diversus,* but much smaller, less than 2.7 mm in length. Signature with broad but variable lateral expansions. For detailed information, see descriptions of subspecies below.

Type-host: *Pelecanoides garnotti* (Lesson).

Discussion: Eichler's description, beyond the fact that a type-host is designated, is virtually useless, and is based on a single female! It is presumed here that the specimens collected from *Pelecanoides garnotti* are the same as his.

Halipeurus (Halipeurus) falsus falsus Eichler
(Figs. 3–7, J-1)

Halipeurus falsus Eichler, 1949, Rev. Brasil. Biol. **9**, No. 3, pp. 338, text figs. 2, 3. Type-host: *Pelecanoides garnotti* (Lesson).

Description: Much smaller, but similar generally to *H. taxosetus* sp. nov. Head ratio 2.0. Signature variable, but generally distinctive in that lateral expansions are large and irregular in outline. Medial indentation variable, usually narrow and straight sided. Abdomen ratio 6.0. Buttresses with well-developed medial processes. Buttress 9 and 10 only lightly sclerotized. Sixth segment only slightly more than one-half as long as the 5th. Setal pattern as in *H. taxosetus,* with small variations, especially in two rows of three closely set setae laterad on sternite 9 and 10. Female with signature like that of male.

Measurements: 10 males, 10 females, from the type-host; averages and ranges in millimeters.

	Males	*Females*
Total length	2.90 (2.70 to 2.93)	3.45 (3.32 to 3.70)
Length of head	0.63 (0.60 to 0.66)	
Width of head	0.32 (0.31 to 0.34)	
Length of abdomen	1.80 (1.69 to 1.85)	
Width of abdomen	0.30 (0.27 to 0.32)	

Type-host: Pelecanoides garnotti (Lesson).

Material examined: All from the type-host as follows: Allotype male, 5 other males and 2 females, from MCZ skin no. 82890, Valparaiso, Chile (RLE); 1 male, 2 females, Valparaiso, Chile, (GBT); 13 males, 13 females, Ancon, Peru, (AMNH).

Halipeurus (Halipeurus) falsus pacificus subsp. nov.
(Figs. 3-7, J-2)

Description: Head ratio 1.9. Signature distinctly modified, with hyaline, 'ear-like' processes laterad of lined area, and broadly expanded, lateral expansions which smoothly curve mediad to a small, rounded medial indentation. Basal segment of antenna relatively short and sub-bulbous; distal two segments barely angled. Abdomen ratio 5.2. Sixth segment two-thirds as long as 5th. Female with distinctive signature.

Measurements: 6 males and 16 females from all hosts; averages and ranges in millimeters.

	Males	*Females*
Total length	2.74 (2.62 to 2.77)	3.16 (3.00 to 3.38)
Length of head	0.61 (0.61)	
Width of head	0.32 (0.31 to 0.34)	
Length of abdomen	1.68 (1.61 to 1.69)	
Width of abdomen	0.32 (0.29 to 0.33)	

Type-host: Pelecanoides urinatrix (Gmelin). See discussion.

Material examined: On various hosts as follows:

Pelecanoides urinatrix (Gmelin): Holotype male, allotype female, paratype male, from AMNH skin no. 528725, New Zealand (RLE). 1 damaged male, 1 female from New Zealand (GBT). 1 male, 1 female, paratypes, New Zealand (RLE).

Pelecanoides magellani (Mathews): 2 male, 10 female paratypes, from southern coast of Chile, (AMNH).

Pelecanoides exsul Salvin: 1 male, 3 females, from AMNH skin no. 211800, collected near Channel Island, New Zealand, (RLE). 1 male and 1 female, from New Zealand, (GBT).

Halipeurus (Halipeurus) thompsoni sp. nov.
(Figs. 3-7, K)

Description: Head ratio 2.1. Signature variable in length, with deep, irregularly rounded median indentation. Small 'ear-like' hyaline extensions of lateral margin anteriorly. Basal segment of antenna relatively short, essentially sub-bulbous; 2d segment enlarged, as broad as basal segment. Abdomen ratio 5.1. Buttresses with medial processes developed. Segment 6 about two-thirds the length of 5. Buttress 9 and 10 relatively long and narrow, curving mediad distally. Segment 11 with moderately sclerotized tergite and buttress, not divided apically. Sternite 9 and 10 with heavily sclerotized posterior margin, overlapping 11th segment. Setal pattern modified, group II setae in two parallel rows. Parameres only lightly sclerotized, slender, subequal. Basal apodeme broad, indistinct. Terminal segments of female reduced slightly. Signature like that of *taxosetus*.

Measurements: 8 males and 4 females from type-host; averages and ranges in millimeters.

	Males	*Females*
Total length	3.46 (3.23 to 3.54)	4.02 (3.93 to 4.08)
Length of head	0.78 (0.73 to 0.80)	
Width of head	0.37 (0.34 to 0.37)	
Length of abdomen	2.12 (2.00 to 2.15)	
Width of abdomen	0.41 (0.37 to 0.44)	

Type-host: Puffinus bulleri Salvin.

Material examined: From the type-host, holotype male, allotype female, 24 male, 21 female paratypes, collected at various places along the coast of Chile by R. H. Beck, (AMNH).

Discussion: This species is named for Mr. Gordon B. Thompson, who has kindly supplied me with considerable material.

This species links *Halipeurus taxosetus* with *H. micariproctus* sp. nov. a larger species with extreme reduction and sclerotization of the 11th segment.

It is easily separated from all other species by the sclerotized modifications of the terminal segments.

Halipeurus (*Halipeurus*) *micariproctus* sp. nov.
(Figs. 3–7, L)

Description: Large. Head ratio 1.85, broad. Signature with pronounced hyaline 'ear-like' processes anteriorly; median indentation broadly rounded. Posterior margin of lateral expansion occasionally irregularly fused to dorsal carina. Basal segment of antenna bulbous, 2nd segment enlarged. Abdomen ratio 4.6, spatulate. Medial processes broadly rounded where present, buttresses 7 and 8 almost straight medially. Segment 6 only slightly shorter than 5. Distal portion of buttress 9 and 10 strongly curved mediad. 11th segment greatly reduced, although heavily and distinctively sclerotized. Sternite 9 and 10 essentially as in *H. thompsoni*, although more heavily sclerotized and extending almost to apex of abdomen. Genitalia heavily sclerotized, with subequal parameres and a caplike sclerotization distad on the aedeagal sac. Basal apodeme indistinct. Female not larger than male, an unusual condition, and with greatly reduced terminal segments; signature without 'ears'.

Measurements: 8 males, 14 females from the type-host; averages and ranges in millimeters:

	Males	*Females*
Total length	4.98 (4.76 to 5.15)	4.90 (4.76 to 5.15)
Length of head	0.91 (0.88 to 0.95)	
Width of head	0.49 (0.48 to 0.51)	
Length of abdomen	3.32 (3.15 to 3.38)	
Width of abdomen	0.72 (0.65 to 0.78)	

Type-host: Puffinus carneipes Gould.

Material examined: From the type-host, holotype male, allotype female, 6 male and 20 female paratypes, from various places around New Zealand (RLE). From other hosts as follows:

Puffinus creatopus Coues: 6 males, 18 females, from MCZ skins collected at Monterey Bay, Calif., and along Chilean coast, (RLE).

Puffinus gravis (O'Reilly): 12 males and 15 females from AMNH skins, collected along the Argentine coast (RLE); 1 male and 2 females from the South Atlantic, (GBT).

Discussion: This species may be readily separated from all others on the basis of its modified terminal segments. It is most unusual in that the females are not markedly larger than the males.

Halipeurus (*Halipeurus*) *attenuatus* sp. nov.
(Figs. 3–7, M)

Description: Head ratio 1.9, broadest above antenna. Signature more-or-less straight-sided, elongated, without a median indentation. Ventral carina indistinct. Basal segment of antenna subcylindric. Abdomen ratio 6.8. All buttresses with only slightly developed medial processes. Segment 6 only one-third the length of 5. Terminal segments elongated, 11th segment with obvious buttress, broadened distally. Sternite 9 and 10 with drawn-out setal pattern. Parameres short, straight and spatulate, subequal. Aedeagal sac almost as long as longest paramere. Female signature squared, as in male; with relatively elongate terminal segment.

Measurements: 7 males, 6 females from the type-host, averages and ranges in millimeters:

	Males	*Females*
Total length	3.52 (3.38 to 3.54)	3.87 (3.85 to 4.00)
Length of head	0.70 (0.68 to 0.73)	
Width of head	0.37 (0.34 to 0.38)	
Length of abdomen	2.18 (2.08 to 2.23)	
Width of abdomen	0.32 (0.29 to 0.34)	

Type-host: Puffinus l'herminieri subalaris Ridgway.

Material examined: Holotype male, allotype female and 3 male, 4 female paratypes, from MCZ skins nos. 112417 and 112418, collected near the Galapagos Islands, from the type-host, (RLE); 5 males, 2 females, paratypes, from type-host, same locality, in Kellogg collection.

Discussion: The type specimens of *Lipeurus diversus* var *major* Kellogg and Kuwana, from the same host, will be discussed under *Halipeurus intermedius* nom.

nov. *H. attenuatus* is closely allied morphologically to *H. mundae* sp. nov., but may be distinguished from it by the shape of the signature and the male genitalia.

Halipeurus (*Halipeurus*) *mundae* sp. nov.
(Figs. 3-7, N)

Description: Unusual louse belonging to *diversus* species group. Head ratio 2.1. Basal segment of antenna almost bulbous. Signature more-or-less typical with indistinct medial indentation. Abdomen ratio 5.4, subspatulate. Medial processes on all buttresses. Sixth segment one-half the length of 5th. Sternite 9 and 10 squared posteriorly, with typical, but slightly drawn out and irregular setal pattern. Parameres short, with spatulate, irregularly margined tips. Aedeagal sac obviously sclerotized, almost as long as longest paramere. Female typical.

Measurements: 2 males, 2 females from the type-host, averages in millimeters:

	Males	*Females*
Total length	3.93	4.08
Length of head	0.78	
Width of head	0.37	
Length of abdomen	2.50	
Width of abdomen	0.45	

Type-host: *Puffinus assimilis mundae* (Salvin).

Material examined: Holotype male, allotype female, 1 male, 1 female, paratypes, from AMNH skin no. 211652, collected by R. H. Beck and J. G. Correia, at 49° S, 179° W, (RLE). *Puffinus assimilis elegans* Giglioli and Salvadori, 2 females, Inaccessible Island, collected by F. H. I. Elliot, 5 July 50.

Discussion: This species represents an unusual development of the *diversus*-type, with short, modified parameres, and enlarged basal segment of the male antenna.

Halipeurus (*Halipeurus*) *turtur* sp. nov.
(Figs. 3-7, O)

Description: A small species closely related to *H. accentor* sp. nov. Head ratio 2.1. Antenna with cylindric basal segment. Abdomen ratio 5.8, relatively narrow. Buttresses IV–VIII with well developed medial processes. Genitalia broad, with parameres essentially symmetrical, tips broadened, spatulate. Obvious and distinctive sclerotization within aedeagal sac, reminiscent of that found in *H. accentor.* Setae 5 and 6 of group II moved to lateral margin of sternite 9 and 10, leaving 1 to 4 joined by the two setae normally anteriad, forming two closely set rows of three setae each. Female with obvious medial indentation in signature.

Measurements: 2 males, 1 female, averages only in millimeters:

	Males	*Females*
Total length	3.38	4.00
Length of head	0.70	
Width of head	0.34	
Length of abdomen	2.16	
Width of abdomen	0.37	

Type-host: *Pachyptila turtur turtur* Kuhl.

Material examined: From type-host, holotype male, allotype female, 1 female paratype, from AMNH skin no. 334593, collected by R. H. Beck, 35° S, 175° W, (RLE).

Discussion: A small distinctive species, that, like *H. bulweria,* appears to be derived from a species found typically on *Puffinus* or *Pterodroma.* In this case, it appears that *H. accentor* from *Pterodroma leucoptera* and *cookii* is the most closely related species.

Halipeurus (*Halipeurus*) *procellariae* (Fabricius)
(Figs. 3-7, P)

Pediculus procellariae Fabricius, 1775, (nec *procellariae* Gurlt (1837), nec *procellariae* Leach MS., Denny (1852) both *nomina nuda*), Systema Entomologiae, Flensburg, p. 808.

Type-host: See discussion below. Considered unrecognizable. Thompson, 1938, Ann. Mag. Nat. Hist., ser. 11, **2**, p. 491.

Miss Theresa Clay, working with Mr. G. H. E. Hopkins, is doing a commendable job revising the early literature of Mallophaga and wishes to place *Pediculus procellariae* Fabricius. The description is brief, translated from Latin as follows: "—a filiform, fuscous, pale footed species on a Brazilian procellariform." While description is apt for *Halipeurus*, and since *Esthiopterum constrictiventre* Pessoa and Guimaraes from *Pterodroma macroptera* is synonymous with *H. diversus* (Kellogg), the louse typically found on this host is undescribed. This louse is also commonly found on four species of *Pterodroma*, namely *mollis, incerta, lessoni* and *macroptera*, that range widely over the South Atlantic making it a very likely possibility. No neotypes will be erected.

Description: Another species of the *H. marquesanus* group. Head ratio 1.9, broad, strikingly 'egg-shaped'. Signature elongate with tapering posterior extension. Premarginal carina not interrupting head outline as usual. Ventral carina distinct. Basal segment of antenna subcylindric, 2d segment relatively large. Pterothoracic patch typical. Abdomen ratio 5.9, barely subspatulate. Buttresses relatively large and with well developed medial processes. Sixth segment one-half the length of the 5th. Sternite 9 and 10 heavily sclerotized, with basic 'T' shape, the shaft of the 'T' expanded and bell-shaped, overlapping the 11th segment slightly. Genitalia broad, parameres relatively short, broadened distally and curving medially, symmetrical. Aedeagal sac about two-fifths the length of parameres. Females typical.

Measurements: 10 males, 8 females from *Pterodroma incerta*, averages and ranges in millimeters:

	Males	*Females*
Total length	4.10 (3.69 to 4.45)	4.60 (4.15 to 4.77)
Length of head	0.82 (0.75 to 0.88)	
Width of head	0.43 (0.37 to 0.47)	
Length of abdomen	2.60 (2.46 to 2.85)	
Width of abdomen	0.44 (0.41 to 0.48)	

Type-host: Pterodroma macroptera (Smith).

Material examined: Pterodroma incerta (Schlegel): 11 males, 9 females, collected 36° S, 46° W, no further data (RLE): 2 males and 1 female, (GBT). Other hosts as follows:

Pterodroma lessoni lessoni (Garnot): 2 males, 4 females, collected by R. H. Beck from the Antipodes Islands, New Zealand, (RLE).

Pterodroma lessoni australis (Mathews): 6 males, 3 females, collected by R. H. Beck from the Antipodes Islands, New Zealand; 1 female, same locality, (GBT).

Pterodroma caribbaea Carte: 2 females, from MCZ skin collected at Jamaica, 1879, (RLE).

Pterodroma macroptera (Smith): 1 male and 1 female, no data, (GBT).

Pterodroma macroptera gouldi (Hulton): 2 males and 2 females, New Zealand, (GBT).

Pterodroma macroptera albani Mathews: 5 males and 1 female, Albany, (GBT).

Pterodroma mollis mollis (Gould): 2 males, 2 females from AMNH skin no. 132496, collected at 36° S, 46° W, (RLE).

Halipeurus (*Halipeurus*) *kermadecense* (Johnston and Harrison) (Figs. 3–7, ♀)

Lipeurus kermadecense Johnston and Harrison, 1912, Tr. & Proc. New Zeal. Inst., **44**, p. 365, fig. 1. Immature. *Type-host: Aestrelata neglecta* Schlegel.

Lipeurus diversus var. *excavatus* Johnston and Harrison, as above, p. 366, fig. 2. *Type-host: Aestrelata neglecta* Schlegel. Adult of *kermadecense*.

Esthiopterum kermadecense (Johnston and Harrison). Harrison, 1916, Parasit., **9**, No. 1, p. 136.

Halipeurus kermadecense (Johnston and Harrison). Thompson, 1938, Ann. Mag. Nat. Hist., ser. 11, **2**, p. 488.

Description: Head ratio 2.1. Signature with medial posterior extension. Well developed ventral carina. Premarginal carina as in *H. procellariae*, narrow and not interrupting outline of head. Antenna relatively long and slender. Abdomen ratio 5.5. Buttresses broad, with well-developed medial processes. Sixth segment about one-half the length of 5th. Terminal segments generally darkened ventrally, but without obvious development seen in closely related *H. procellariae*. Genitalia broad, parameres with spatulate tips, only slightly subequal, not as symmetrical as in procellariae. Tube-like sclerotization within aedeagal sac, obliquely cut distally. Setal pattern typical. Signature of female squarer than in male but with similar outline.

Measurements: 10 males, 10 females from various hosts, averages and ranges in millimeters:

	Males	*Females*
Total length	3.66 (3.54 to 3.70)	4.36 (4.24 to 4.62)
Length of head	0.77 (0.75 to 0.82)	
Width of head	0.37 (0.34 to 0.39)	
Length of abdomen	2.22 (2.15 to 2.31)	
Width of abdomen	0.40 (0.37 to 0.44)	

Type-host: *Pterodroma phillipii* Gray.

Material examined: From the type-host: 4 males, 5 females, from MCZ skin no. 82907, Masa Tierra Island, (RLE); 8 males, 3 females, no locality, (GBT). From other hosts as follows:

Pterodroma arminjoniana (Giglioli and Salvadori): 2 males, 2 females, from South Trinidad Island, (Meinertzhagen); 1 male, 2 females, South Trinidad Island, (GBT).

Pterodroma externa externa (Salvin): 2 males, from MCZ skin no. 82905, Masa Tierra Island, (RLE).

Discussion: The classification of the genus *Pterodroma* used by Peters (1931) needs radical revision; it is used here for the sake of uniformity.

The specimens from *P. externa* listed above are probably stragglers, this host normally is infested with *H. intermedius* nom. nov.

H. kermadecense may be separated from *H. procellariae* by virtue of the similar, but distinctly asymmetrical parameres.

Halipeurus (*Halipeurus*) *accentor* sp. nov.
(Figs. 3–7, R)

Description: Head ratio 2.1, relatively narrow. Signature more-or-less typical of *Pterodroma* species, extended posteriorly with slight medial indentation. Barely distinct ventral carina. Pterothoracic setal patch with three closely set setae. Abdomen ratio 6.0, relatively straight sided. Sixth segment about one-half as long as 5th. Sternite 9 and 10 overlapping 11th with rounded posterior border. Sclerotized laterally, superficially resembling *H. forficulatus* sp. nov. Setal pattern typical. Genitalia broad with moderately sclerotized basal apodeme. Parameres subequal, spatulate and pointed, variable depending on orientation within body. Aedeagal sac about three-fourths the length of parameres. Females typical.

Measurements: 8 males, 7 females, from various hosts, averages and ranges in millimeters:

	Males	*Females*
Total length	3.78 (3.69 to 3.92)	4.15 (3.99 to 4.30)
Length of head	0.72 (0.68 to 0.75)	
Width of head	0.35 (0.34 to 0.37)	
Length of abdomen	2.42 (2.31 to 2.62)	
Width of abdomen	0.40 (0.37 to 0.42)	

Type-host: *Pterodroma leucoptera masafuerae* Lonnberg.

Material examined: From the type-host, holotype male, allotype female, 7 male and 5 female paratypes, from AMNH skin no. 446141, collected near Masa Tierra Island, Chile, (RLE). Other material as follows:

Pterodroma leucoptera hypoleuca (Salvin): 1 male, 1 female, paratypes, from the Bonin Islands, (GBT).

Pterodroma cookii nigripennis (Rothschild): Paratype female from MCZ skin no. 39895, collected near Kermadec Islands, (RLE); 3 male, 3 female paratypes, Kermadec Islands, (GBT).

Pterdroma cookii difilipianna (Giglioli and Salvatori): 3 paratype males from MCZ skin no. 82092, collected near Masa Tierra Island, (RLE); male and female, 80 miles west Ancon, Peru, (Curran-AMNH).

Halipeurus (*Halipeurus*) *intermedius* nom. nov.
(Figs. 3–7, S)

Lipeurus diversus var. *major* Kellogg and Kuwana, 1902, (nec Piaget, 1880) Proc. Wash. Acad. Sci., 4, p. 477. Type-host: *Puffinus subalaris.*

Halipeurus sp?. Thompson, Ann. Mag. Nat. Hist., ser. 11, **2**, p. 485.

Description: Head ratio 2.1, narrow. Signature squarish, posterior extension narrowed. Premarginal carina barely separated from post-marginal carina. Basal segment of antenna essentially cylindric, but very wide, 2d and 3d segments enlarged relatively. Pterothoracic patch divided. Abdomen ratio 6.2. Buttress with posterior medial processes well developed, more or less pointed. Segment 6 only slightly smaller than 5. Sternite 9 and 10 with faint 'T' shaped sclerotization, slightly overlapping 11. Setal pattern typical but variable. Eleventh segment short and triangular, with narrow buttresslike sclerotization. Genitalia broad with long distinct basal apodeme. Parameres moderately long, broad, sub-equal, with tips pointed and turned mediad slightly. Aedeagal sac about three-fourths length of parameres. Female with terminal segments as in *H. marquesanus* and *postmarquesanus*.

Measurements: 6 males, 5 females from hosts listed:

	Males	*Females*
Total length	4.82 (4.62 to 4.92)	4.94 (4.85 to 5.00)
Length of head	0.81 (0.78 to 0.85)	
Width of head	0.38 (0.38 to 0.41)	
Length of abdomen	3.16 (3.08 to 3.23)	
Width of abdomen	0.51 (0.49 to 0.52)	

Type-host: Pterodroma phaeopygia phaeopygia (Salvin).

Material examined: From the type host, holotype male, allotype female, from AMNH skins collected by R. H. Beck from the Galapagos (RLE); 4 paratype males from MCZ skin no. 65683, Galapagos (RLE); male paratype, Galapagos (GBT).

Pterodroma externa cervicales (Salvin): 2 males paratypes, collected by R. H. Beck near Sunday, Kermadec Islands (AMNH); 3 male, 3 female paratypes, Kermadec Islands (GBT); 3 male, 5 female paratypes, from MCZ skin no. 39893, Kermadec Islands (RLE).

Puffinus subalaris Ridgway: 1 male, 1 female, syntypes of Kellogg and Kuwana's *Lipeurus diversus* var. *major*, (these are, without doubt, stragglers).

Discussion: These species may be separated from the other closely related species, *H. marquesanus* and *postmarquesanus* by the genitalia and chaetotaxy of the male terminal segments.

Halipeurus (*Halipeurus*) *marquesanus* (Ferris)
(Figs. 3-7, T)

Esthiopterum marquesanus Ferris, 1932, Bull. Bishop Mus. Honolulu, **98**, pp. 62-63, figs. 14, 15. *Type-host: Pterodroma rostrata. Halipeurus marquesanus* (Ferris). Thompson, 1938, Ann. Mag. Nat. Hist., ser. 11, **2**, p. 489.

Description: Head ratio 2.1, narrow. Signature relatively short, with prolonged medial portion posteriorly. Pre- and postmarginal carinae more-or-less fused. Dorsal carinae extend to level of mandibles in male, but are much shorter in female. Pterothoracic patch atypical in male, usually in two parts, one part with a single long seta, the other with two setae. Abdomen ratio 7.3, exceptionally narrow, straight-sided. Sixth segment slightly smaller than 5th. Buttresses relatively narrow with only moderately developed medial processes. Sternite 9 and 10 with rectangular extension posteriorly, overlapping segment 11. Setal pattern of group II crowded, slightly distorted, with setae 1 and 2 close set, and 5 and 6 moved anteriorly. Parameres long and slender, with slightly broadened tips. Aedeagal sac about two-thirds as long as parameres. Sternite 9 of female expanded and extended as an obvious loose flap over next segment. Buttresses 9 and 10 and 11 smoothly fused, as same tergites almost are.

Measurements: 5 males and 8 females from the type-host, averages and ranges in millimeters:

	Males	*Females*
Total length	4.00 (3.85 to 4.16)	4.46 (4.15 to 4.62)
Length of head	0.74 (0.71 to 0.75)	
Width of head	0.34 (N.V.)	
Length of abdomen	2.62 (2.62 to 2.69)	
Width of abdomen	0.36 (0.34 to 0.37)	

Type-host: Pterodroma rostrata rostrata (Peale).

Material examined: 1 male, 2 female paratypes kindly loaned by Mr. Gordon B. Thompson;

26 males, 29 females, from MCZ skin no. 166885, from the Society Islands, (RLE), all from type-host.

Halipeurus (Halipeurus) postmarquesanus sp. nov.
(Figs. 3–7, U)

Description: Very close to *H. marquesanus.* Head ratio 2.0. Pre- and postmarginal carinae not fused. Pterothoracic patch variable, usually divided into two groups. Abdomen ratio 6.8, very similar to *H. marquesanus* except for the elongated segment 9 and 10. Group II setae crowded posteriorly. Parameres and basal apodeme forming very long, narrowly 'S' shaped unit. Female with terminal segments like those of *H. marquesanus.*

Measurements: 5 males and 6 females from the type-host, averages and ranges in millimeters:

	Males	*Females*
Total length	3.72 (3.62 to 3.85)	4.30 (4.15 to 4.38)
Length of head	0.68 (0.65 to 0.70)	
Width of head	0.34 (0.32 to 0.35)	
Length of abdomen	2.43 (2.32 to 2.46)	
Width of abdomen	0.36 (0.34 to 0.37)	

Type-host: Pterodroma heraldica (Salvin).

Material examined: Holotype male, allotype female, 3 male, 4 female paratypes, from AMNH skin collected in the South Pacific, (RLE); 1 male and 2 female paratypes, Chesterfield Group, (GBT).

Pterodroma parvirostris (Peale): 1 male, 1 female, no data, (GBT).

Discussion: Readily separated from both *H. marquesanus* and *intermedius* by the distinctly shaped male genitalia.

List of the Species and Subspecies of Halipeurus (Halipeurus) *and Their Hosts*
(* *Type-Host*)

H. angusticeps angusticeps (Piaget)
Host unknown.
H. a. fosteri subsp. nov.
* *Puffinus leucomelas* (Temminck)
H. abnormis (Piaget)
* *Puffinus gravis* (O'Reilly)
Puffinus kuhlii kuhlii (Scopoli)
Puffinus kuhlii borealis Cory
Puffinus kuhlii edwardsi Oustalet
Puffinus kuhlii flavirostris (Gould)
H. mirabilis Thompson
Puffinus pacificus pacificus (Gmelin)
* *Puffinus pacificus chlorohynchus* Lesson
Puffinus pacificus cuneatus Salvin
H. placodus sp. nov.
Puffinus assimilis assimilis Gould
* *Puffinus assimilis tunneyi* Mathews
Puffinus assimilis kermadecensis Murphy
Puffinus l'herminieri nugax Mathews
Puffinus auricularis Townsend
H. diversus (Kellogg)
* *Puffinus griseus* (Gmelin)
Puffinus tenuirostris (Temminck)
Puffinus puffinus puffinus (Brunnich)
Puffinus puffinus yelkouen (Acerbi)
Puffinus puffinus mauretanicus Lowe
H. forficulatis sp. nov.
* *Puffinus l'herminieri polynesiae* Murphy
Puffinus l'herminieri dichrous Finsch and Hartlaub
Puffinus heinrothi Reichenow

H. bulweriae sp. nov.
* *Bulweria bulweria* (Jardine and Selby)
H. taxosetus sp. nov.
* *Puffinus nativitatis* Streets
Puffinus gavia Forster
Puffinus opisthomelas Coues
Puffinus l'herminieri l'herminieri Lesson
Puffinus l'herminieri bannermani Mathews & Iredale
Puffinus l'herminieri bailloni (Bonaparte)
Puffinus assimilis baroli (Bonaparte)
H. falsus falsus Eichler
* *Pelecanoides garnotti* (Lesson)
H. f. pacificus subsp. nov.
* *Pelecanoides urinatrix* (Gmelin)
Pelecanoides magellani (Mathews)
Pelecanoides exsul Salvin
H. thompsoni sp. nov.
* *Puffinus bulleri* Salvin
H. micariproctus sp. nov.
* *Puffinus carneipes* Gould
Puffinus creatopus Coues
Puffinus gravis (O'Reilly)
H. attenuatus sp. nov.
* *Puffinus l'herminieri subalaris* Ridgway
H. mundae sp. nov.
* *Puffinus assimilis munda* (Salvin)
Puffinus assimilis elegans Giglioli and Salvadori
H. turtur sp. nov.
* *Pachyptila turtur turtur* (Kuhl) (Tentative)
H. procellariae (Fabricius)
Pterodroma incerta (Schlegel)
Pterodroma lessoni lessoni (Garnot)
Pterodroma lessoni australis (Mathews)
Pterodroma caribbaea Carte
* *Pterodroma macroptera macroptera* (Smith)
Pterodroma macroptera gouldi (Hutton)
Pterodroma macroptera albani Mathews
Pterodroma mollis mollis (Gould)
Pterodroma inexpectata (Forster)
H. kermadecense (Johnston and Harrison)
* *Pterodroma phillipii* (Gray)
Pterodroma arminjoniana (Giglioli and Salvadori)
Pterodroma externa externa (Salvin)
H. accentor sp. nov.
* *Pterodroma leucoptera masafuerae* Lonnberg
Pterodroma leucoptera hypoleuca (Salvin)
Pterodroma cookii nigripennis (Rothschild)
Pterodroma cookii difilipianna (Giglioli and Salvadori)
H. intermedius nom. nov.
* *Pterodroma phaeopygia phaeopygia* (Salvin)
Pterodroma externa cervicalis (Salvin)
H. marquesanus (Ferris)
* *Pterodroma rostrata rostrata* Peale
H. postmarquesanus sp. nov.
* *Pterodroma heraldica* (Salvin)
Pterodroma parvirostris (Peale)

Subgenus Synnautes *Thompson*

Synnautes Thompson, 1936, Ann. Mag. Nat. Hist., ser. 10, **18,** p. 43. Genotype: *Lipeurus pelagicus* Denny.
Naubates Bedford, *partim.* T. Clay, 1940, Brit. Graham Land Exped., 1911–14, ser. C, **2,** pt. 1, p. 31.

Description: Similar to subgenus *Halipeurus,* differing as follows: Buttresses of abdomen

very narrow, with medial processes limited to small, knob-like enlargements at posterior ends of buttresses. Ventral carina usually heavily sclerotized; dorsal carina typically extending only to mid-point between signature and mandibles (exception *nesofregettae* sp. nov.). Pre- and postmarginal carinae more or less fused; premarginal carina obliquely truncate anteriorly, interrupting outline of head (fig. 6). Female abdomen sharply bifid, always with very stout, short seta terminating each buttress of segment 11. Cuticle obviously scaly, more so than in subgenus *Halipeurus*.

Type of subgenus: Halipeurus (Synnautes) pelagicus (Denny) 1842.

Discussion: The species of subgenus *Synnautes* are limited to birds of the family *Hydrobatidae*. They seem best adapted for living on the back and proximal portions of the wings, as are species of subgenus *Halipeurus*.

The new species herein described is the second, and several more remain to be described. The subgenus has species that reflect many of the variations of subgenus *Halipeurus*, indicating that considerable parallel evolution has taken place between the two subgenera.

Halipeurus (Synnautes) pelagicus (Denny)
(Figs. 3–7, V)

Lipeurus pelagicus Denny, 1842, Mono. Anoplurorum Brit., pp. 173–174, pl. 14, fig. 2. *Type-host: Thalassodroma pelagica* (cf. Thompson, 1937, on the Denny collection).
Lipeurus subangusticeps Piaget, 1880, Les Pediculines, pp. 308–309, pl. 25, fig. 5. *Type-host: Thallassodroma leachi* (from Rotterdam Zoo).
Lipeurus languidus Kellogg and Kuwana, 1902, Proc. Wash. Acad. Sci., **4**, pp. 475–476, pl. 29, fig. 8. *Type-hosts: Oceanodroma tethys* and *Oceanites gracilis*.
Lipeurus exiguus Kellogg and Kuwana, as above, p. 479, pl. 30, fig. 2. Immatures. *Type-host: Oceanites gracilis*.
Esthiopterum pelagicum (Denny). Harrison, 1916, Parasit., **9**, No. 1, p. 139.
Synnautes pelagicus (Denny). Thompson, 1936, Ann. Mag. Nat. Hist., ser. 10, **18**, p. 43. Genotype of genus *Synnautes* Thompson.

Description: As for subgenus, further details as follows: Slender, very dark lice, widely distributed on *Hydrobatidae*. Head ratio 2.2, very slender, with nearly parallel sides. Signature triangular anteriorly, with very broad hyaline border, straight-sided, with tapered posterior extension and small, narrow medial indentation. Thorax with narrow buttresses. Abdomen ratio 5.0, very slender. Tergites evenly sclerotized, with band narrowed slightly only on segments 6, 7, and 8. Buttresses very small, best developed on ends of buttresses of segments 6, 7, and 8. Buttresses 9 and 10 smoothely tapered. Sternite 9 and 10 distinctly sclerotized, with setal pattern similar to that of *H. (Halipeurus) taxosetus*, with slight modification. Parameres long, slender, flattened, and somewhat irregularly curved from specimen to specimen. Female typical of subgenus, with signature very much like that of male.

Measurements: 5 males, 3 females, from *Pelagodroma m. marina*, averages and ranges in millimeters as follows:

	Males	*Females*
Total length	3.52 (3.32 to 3.63)	4.03
Length of head	0.78 (0.75 to 0.82)	0.80
Width of head	0.37 (0.34 to 0.39)	0.45
Length of abdomen	2.14 (2.02 to 2.24)	2.67
Width of abdomen	0.42 (0.37 to 0.44)	0.54

Type-host: Hydrobates pelagicus (Linné).

Material examined: From the type-host, 2 males, 1 female, from MCZ skin no. 235247, Basket Islands, Ireland (RLE). Other material as follows:

Bulweria bulweria (Jardine and Selby): 2 females, from MCZ skin no. 98117, collected near Madeira Island, (RLE). Stragglers?

Oceanites oceanicus oceanicus (Kuhl): 2 females, mar del Plata, Argentina, (AMNH).

Oceanites gracilis gracilis (Elliot): 1 male, 4 females, from AMNH skin no. 206965, collected near Pacas Mayo, Peru, (RLE).

Oceanites gracilis galapagoensis Lowe: 1 female, from Galapagos Islands, (AMNH).

Pelagodroma marina marina (Latham): 4 males, 3 females, from AMNH skin no. 254335, from Herald Islands, New Zealand, (RLE); 5 males, 6 females, from skin in Preparations Department of AMNH, collected at Tristan da Cunha.

Pelagodroma marina maoriana Mathews: 2 females from AMNH skin, no further data, (RLE); 7 males, 6 females, from 37° S–179° W, (AMNH).

Fregetta grallaria grallaria (Vieillot): 2 males, 4 females, from AMNH skin no. 446402, collected near Masa Tierra Island, Chile, (RLE); 8 males, 8 females, from Masa Tierra Island, Chile, (AMNH).

Fregetta grallaria titan Murphy: 1 male, from Rapa Island, (AMNH).

Fregetta tropica melanogaster (Gould): 1 male, 2 females, from AMNH skin no. 212047, 49° S–179° W, (RLE).

Oceanodroma tethys tethys (Bonaparte): 3 males, from AMNH skin no. 196377, 2° S–91° W, (RLE). 3 males, 5 females, from MCZ skin no. 111806, Galapagos Islands, (RLE).

Oceanodroma castro castro (Harcourt): 3 males, 2 females, from MCZ skin no. 300905, from Porto Santo, Madeira, (RLE).

Oceanodroma castro bangsi Nichols: 1 female, Galapagos, (AMNH).

Oceanodroma leucorhoa keadingi Anthony: 1 male, Guadalope Island, (AMNH).

Oceanodroma macrodactyla Bryant: 1 male from AMNH skin, no data, (RLE).

Oceanodroma markhami tristrami Salvin: 1 female, from Hawaiian Islands, (AMNH).

Oceanodroma hornbyi (Gray): 1 male, from 80 miles west of Ancon, Peru, (AMNH).

Oceanodroma furcata (Gmelin): 1 female, from MCZ skin no. 321672, collected near Sitka, Alaska, (RLE).

Halocyptena microsoma Coues: 1 damaged male, from MCZ skin no. 101692, from San Benito Island, (RLE); 1 female from AMNH skin, same locality, (RLE).

Discussion: *Synnautes* species are closely related to the *procellariae* species group of *Halipeurus*, as evidenced by their general structure, sclerotization of terminal segments, shape of head, type of signature and male genitalia.

Synnautes pelagicus is notable in that it may be found on so many different host species. The other two species of *Synnautes* in my collection, *nesofregettae* and one undescribed, are each restricted to one host species.

Halipeurus (*Synnautes*) *nesofregettae* sp. nov.
(Figs. 3–7, W)

Description: Typical *Synnautes* with some features of typical *Halipeurus*. Head ratio 2.2, narrow, with straight-sided lateral margin. No hiatus between pre- and postmarginal carinae. Dorsal carinae extend to level of mandibles and then unite medially. Ventral carina very distinct. Signature squarish, with posterior extension like that of species of *Halipeurus* from *Pterodroma*. Antenna with long cylindric basal and 2d segment. Pterothoracic patch typical. Abdomen ratio 5.0. Buttresses very narrow, with only slight medial processes, restricted to ends of buttresses. Sixth segment three-fourths as long as 5th. Sternite 9 and 10 overlapping most of 11th segment, with irregular, atypical setal pattern. Parameres moderate in length and breadth, distinctly sub-equal.

Measurements: 5 males, 3 females from type-host, averages and ranges in millimeters:

	Males	*Females*
Total length	3.85 (3.77 to 3.93)	4.16 (4.08 to 4.24)
Length of head	0.83 (0.80 to 0.85)	
Width of head	0.37 (0.36 to 0.38)	
Length of abdomen	2.37 (2.31 to 2.46)	
Width of abdomen	0.47 (0.43 to 0.48)	

Type-host: *Nesofregetta albigularis* (Finsch).

Material examined: From the type-host: Holotype male, allotype female, 4 male and 2 female paratypes, from AMNH skin no. 205901, collected at Phoenix, Phoenix Group, (RLE); 1 female, paratype, from New Hebrides, (GBT).

Discussion: This species closely unites the taxonts *Synnautes* and *Halipeurus*, (cf. Clay 1940, pp. 309–310). It resembles *Halipeurus* in that the dorsal carina definitely extends to the level of the mandibles, and in that the pre- and postmarginal carinae are not united. The female, however, has the typical *Synnautes* terminal segments, with the broadened seta apically on each 11th buttress.

SUMMARY

The genus *Halipeurus,* typically parasitizing the shearwaters and petrels, is revised. Twelve new species and two new subspecies are described. All previously described species are redescribed and refigured and, where necessary, lectotypes designated. Host-parasite relationships are discussed.

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RESEARCH NOTE

A SMALL CULTURE DISH AND AN INVERTED DISSECTING MICROSCOPE FOR IN VITRO CULTURE WORK.

Optically superior cell culture chambers of different designs and varying in complexity have been described from time to time. Two recent reports on this subject are those of Cruickshank, Cooper, and Conran (1959, Exp. Cell Res. **16:** 695–698) and Sykes and Moore (1960, Rept. Biol. Med. **18:** 288–297). Fairly comprehensive references on various designs can be obtained from these two papers. Other versions of cell culture chambers not included in the above references were described by Paul (1959) in his book "Cell and Tissue Culture."

In our work on in vitro cultivation of protozoan parasites whether alone or with host tissue, we have developed a small petri dish that has the following features: (1) Small size (0.2 to 0.6 ml medium capacity) for easy scanning of its contents; (2) convenience for changing medium or transferring the cultured organisms; (3) suitability for maintaining a desired gas phase by confining the dishes in a gassed chamber; (4) optical superiority for oil-immersion photomicrography when used with the aid of an inverted compound microscope; (5) resistance to the rigors of acid baths and autoclaving. Only prolonged soaking in detergents seems to affect the integrity of the dish.

The construction of the dish is very simple. Pyrex culture tubes of two different sizes (i.e., 18 mm and 22 mm outside diameter) were cut into sections on a carborundum wheelblade. The smaller diameter tube was cut into 8-mm sections and the larger diameter tube into 5-mm sections. The cut surfaces were polished on the wheelblade. Coverslips of No. 1 thickness corresponding to the diameters of the respective sections were used for the bottom and top surfaces of the dish. "Sealit" (Fisher Scientific Co.) was used for sealing the coverslip and the section together. The cement was found not to be toxic for either microorganisms or cells in culture.

For phase photomicrography, shallower dishes made of thin stainless-steel sections and coverslips were used. Dishes about 1.8 mm high were found to be most satisfactory. By carefully placing a drop of medium in the center of the bottom dish and another drop on the inner surface of the cover, a column of medium was formed when the dish was assembled.

To facilitate the observation of large microorganisms cultured in these dishes, a device was developed for converting a stereoscopic microscope for inverted use. Such an inverted microscope allows considerable freedom in handling the organisms particularly at high magnification, due to the absence of an obstructing objective housing. In addition, it overcomes the difficulty of obscured vision resulting from moisture condensing on the underside of the upper lid of the dish.

For our purpose, we chose a stereoscopic microscope with a fixed working distance as this could be converted without permanent alteration. The base was removed and the microscope bolted to a piece of heavy angle iron so that it rested on its back at 90 degrees from its normal position (fig. 1). The ocular housing was rotated 180 degrees. A steel tube with an inside

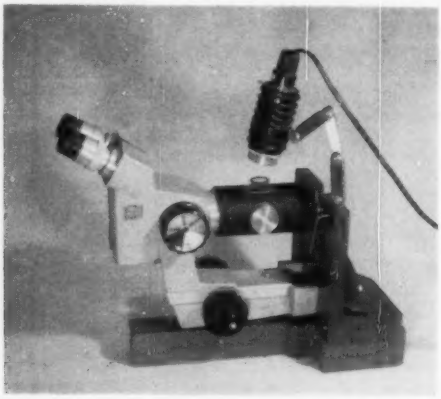

FIGURE 1. Inverted dissecting microscope.

diameter slightly larger than the objective housing was attached to the angle iron in such a way that the objective could be moved back and forth inside the tube for focusing. A front surfaced mirror or prism was placed in the tube at an angle of 45 degrees directly beneath an opening cut into the tube. A piece of glass placed over the opening served as a stage.

The mirror housing and the inside of the steel tube were painted flat black to eliminate glare. With available supplementary lenses, a magnification of 80 power may be obtained.

We wish to express our appreciation to Mr. A. Klain for his help in the design and construction of this equipment.

This work was supported by Grant (E87) to Dr. G. H. Ball from the National Institutes of Health by U. S. Public Health Training Grant (2E-70) in Microbiology to J. Moore.—
JOWETT CHAO AND JOSEPH MOORE, *Department of Zoology, University of California, Los Angeles.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Fiftieth Council Meeting, Los Angeles, California, November 1, 1960

The meeting was called to order at 2:25 P.M. on Tuesday, November 1, in the conference room of the Biltmore Hotel, Los Angeles, California. The meeting was attended by President H. W. Brown, Vice-President P. E. Thompson, President-Elect J. M. Andrews, Past Presidents A. C. Walton, G. F. Otto, and N. R. Stoll, the Secretary, Treasurer M. J. Ulmer, Editor E. R. Becker, Custodian W. S. Bailey, Councilors D. V. Moore, R. M. Lewert, R. V. Bangham, L. J. Olivier, G. H. Ball, D. A. Porter, L. Jachowski, D. T. Clark and M. Voge and by committee members, Chairmen or Delegates L. A. Stauber, L. J. Thomas, N. D. Levine, G. W. Hunter III, K. C. Kates, E. E. Lund, M. M. Brooke and J. S. Andrews.

I. Reports of Officers

1. *President* (*H. W. Brown*): No report.

2. *President-Elect* (*J. M. Andrews*): No report.

3. *Vice-President* (*P. E. Thompson*): No report.

4. *Secretary* (*F. J. Kruidenier*): As of November 1, 1960, there were 1,126 names on the current rolls of the Society distributed as follows: 1,048 Active Members in good standing, 8 non-subscribing Inactive Members, 22 delinquent for 1958, 1959, and 1960 dues (and will be dropped as of January 1, 1961), 27 delinquent for 1959 and 1960 dues, 9 Emeritus Members, and 14 Honorary Members. Notice of the decease of Charter Members Dr. Paul S. Bartsch and Dr. Thurlow C. Nelson have been received. One member resigned as of January 1961. Thirty-three members were dropped from the rolls of the Society in October 1959 for non-payment of 1957, 1958 and 1959 dues.

The report was accepted and ordered placed on file.

5. *Treasurer* (*M. J. Ulmer*): The accompanying summary of the Treasurer's report is for the period of August 1, 1959 to July 31, 1960 in accordance with Council recommendation that the fiscal year of the Society be standardized rather than vary with the date of the annual meeting.

a.

Balance on hand, August 1, 1959	$ 8,694.98
Collections, August 1, 1959 to July 31, 1960	21,929.87
Total	$30,624.85
Expenditures, August 1, 1959–July 31, 1960	21,994.15
Total Cash Balance, August 1, 1960	$ 8,630.70

b. Treasurer Ulmer's report on the Endowment Fund for the period August 1, 1959 to July 31, 1960:

	1/8/59	Interest	7/31/60
Savings Account	$ 515.26	$19.49	$ 534.75
U.S. # M1 681 662F Savings Bond Value	914.00	31.00	945.00
	$1,429.26	$50.49	$1,479.75

The report was accepted subject to audit by the Auditing Committee and ordered placed on file.

6. *Editor* (*E. R. Becker*): Two hundred twenty-five manuscripts were received between August 1, 1959 and July 31, 1960, 144 being regular papers and 81 research notes. With the additional manuscripts (92 regular papers and 29 research notes) received prior to August 1, 1959, a total of 346 manuscripts (110 research notes and 236 full reports) were handled during the fiscal year. Of these, 279 have been processed and 69 are being reviewed or have been returned to their authors for revision. Of those processed, 260 were accepted for publication, 16 rejected, and 3 withdrawn by authors after requests for revision; 142 have been published and 118 await publication.

Approximately 16.7 percent of the reports dealt with protozoa, 71.2 percent with helminths, 9.8 percent with arthropods; and 2.3 percent were general or mixed in subject matter. Nos. 4, 5, and 6 of volume 45 (1959) of the Journal of Parasitology and Nos. 1, 2, and 3 of volume 46 (1960) cost (Business Press Inc.) $2,353, $1,988, $2,734, $2,538, $2,925, and $2,269, respectively.

The December 1960 issue will have about 240 pages with an approximate total of 900 pages in volume 46. This, with the 47-page supplement, is an increase of about 200 pages over the 1950–1959 average.

The increasing value of the Journal makes it desirable that the Council consider the feasibility of copyrighting the publication.

Council directed the Secretary to proceed with the copyrighting for the Journal of Parasitology. The Editor's report was accepted and ordered placed on file.

7. *Custodian of Back Issues* (*W. S. Bailey*):

a. Balance on Hand, (1 August, 1959)	$ 7,574.02*
Net Collections, 1 August, 1959–31 July, 1960	3,939.52
Total Credit, 1 August, 1959–31 July, 1960	$11,513.54
Expenditures, 1 August, 1959–31 July, 1960	3,409.26
Balance on Hand as of 31 July, 1960	$ 8,104.28*
b. Summary of the operation of the Custodian 1954–1960:	
Sale of Back Issues (Average: $2,687)	$18,809.00
Cost for duplication of Back Issues	6,821.00
Payment of Loan for Earlier Duplication	2,000.00
Contributions to Society for Duplications	1,450.00
Operating Costs (average, $435 per year)	3,050.00
Net Credits (1954–1960)	$ 8,388.00
c. INDEX II Finances	
Expenditure to Date	$ 4,869.00
*Approximate Additional Costs (Estimated)	5,200.00
	$10,069.00

It is not possible to continue the sale of INDEX II to members or non-members of the Society at the pre-publication price of $3.75. It is recommended that the Index be sold to new members for $5.00 at the time they become Active Members. It is further recommended that a price of $7.50 be established as the post-publication price to all but new members.

d. Possibilities for Future Operations or Disposal of Inventory.

(a). Continue present program. It will be necessary to establish a more realistic price for back issues if this Society investment is to support other activities.

(b). Contract for Storage, Shipping and Business Operations.

(1). Through Business Press (Publisher of the Journal): Charges for storage ($1.40/M: $46.00/month), insurance ($20.00/year) and actual handling (possibly $400–$500/year) would have to be paid from income.

(2). Through another business agency: If the Society will continue to provide back issues, this agency would store and ship the stock and return the Society 50 percent of the total sales income.

(c). Sell all stock and a specified number of copies of each issue. All duplicating could be done by an agency. The purchase price would be about $6,000 plus routine extra-copy sales. Index II could be retained by the Society or sold at a profit which would be about 50 percent of the purchasing company's net on the sales.

(d). Authorize full page reproductions of out-of-print issues by University Microfilms Inc. The Society would receive 10 percent royalties.

Council established a post-publication price of $5.00 to all new Members and $7.50 to all other Members.

Council asked that an expression of appreciation for her valuable work on Index II be sent to Miss Mildred Doss.

The Custodian was asked to continue his study of the future handling of the back issues.

The report was accepted subject to audit and ordered placed on file.

II. Reports of Standing Committees

1. Committee on Visual Education (M. S. Ferguson, Chr.; E. K. Markell, D. V. Moore, J. C. Schwartzwelder, M. Voge): The revision of the catalogue of films on parasitological subjects, in preparation for the past two years, should be completed early in 1961.

2. Committee on Nomenclature and Terminology (H. W. Manter, Chr.; N. R. Stoll, Consultant; A. McIntosh, Adviser; G. W. Hunter, III, L. R. Penner, G. W. Wharton, F. J. Kruidenier): The new, revised rules of nomenclature should be available very soon.

3. Committee on Awards (L. A. Stauber, Chr.; P. C. Beaver, N. D. Levine, C. P. Read): No recipient was recommended in 1960. Council directed that a notice requesting nominations

*Approximately $5,200 will be required to complete the production of INDEX II. Replacement of 9 out-of-print issues will require $1,285. Thus the total amount pledged is $6,485.

to the H. B. Ward Medal be distributed with the annual notices of dues.

4. Committee on Honorary and Emeritus Members (L. J. Thomas, Chr.; G. H. Ball, L. Jachowski, I. Kagan): In accordance with the limitations imposed by the By-Laws of the Society, the Committee recommended Dr. Emile Roubaud for Honorary Member, and Drs. Willard W. Wright and Henry E. Meleney for Emeritus Members.

Council approved and ordered that the nominations be presented to the Society.

5. In Memoriam Committee (J. E. Ackert, Chr.; H. W. Stunkard, E. Chernin, W. S. Bailey): Dr. Paul S. Bartsch and Dr. Thurlow C. Nelson, Charter Members of the Society, have passed away since the last meeting of the Society.

The deep sense of loss to the Society was expressed by members of Council. Council directed that an In Memoriam be published honoring Drs. Bartsch and Nelson.

6. Tellers' Committee (D. A. Porter, Chr.; K. L. Hussey): A total of 111 ballots were submitted to the Tellers' Committee by Secretary Kruidenier. The tabulation was reported to the Nominations Committee and is reported to Council.

7. Nominating Committee (L. E. Rozeboom, Chr.; Council): A slate of nominees to Society offices has been prepared from the ballots of the members and with regard to the burdens of duties presently assigned to incumbent officers of the Society. It will be presented to the Society for action during the Annual Business Meeting.

8. Resolutions Committee (R. V. Bangham, Chr.; M. Voge): A report of the Resolutions Committee will be presented to the Society at the Annual Business Meeting.

The reports of the Standing Committees were accepted and ordered placed on file.

III. Reports of Representatives and Delegates

1. *To Council of the A. A. A. S.* (G. W. Wharton, E. Sadun, Alt.): An expansion of the authority and responsibility of the Council of the A. A. A. S. is being studied to give affiliated societies more participation. It is recommended that representatives be appointed for at least three years to stabilize the proposed responsibility.

The A. A. A. S. officially recommends the granting of federal research funds on an uncommitted basis to applicant institutions, as well as the present system of project-type grants. The institutional, uncommitted grant would provide a better continuity of research support than is currently available.

2. *To the Governing Board, AIBS* (A. C. Walton, L. Olivier, Alt.): A meeting of the Governing Board was held in May, 1960, at the University of Colorado in Boulder and the Annual Meeting was held in August at Stillwater, Oklahoma. The following officers were elected: President, Dr. Tracy Sonneborn (Zoology, Indiana University); Vice-President, Dr. Bernard Meyer (Botany, Ohio State University); Secretary-Treasurer, Dr. Frank Cullinan (U.S.D.A.); Executive Committee, Dr. Dorothy Matala (Iowa State Teacher's College).

The AIBS is operating on a budget of nearly 2.5 million dollars with total expenses of $240,000 of which only $41,000 is derived from dues. The AIBS has advised various governmental agencies and is active in obtaining financial aid for U. S. and foreign scientists attending national and international meetings.

In addition AIBS has (1) instigated a news service with the "Newsletter" sent out bimonthly to all members, (2) increased the Bulletin to five issues per year (plus the program number), (3) increased the program of translations of Russian journals and started a translation service of Chinese journals, (4) increased redactory and business services, (5) sponsored a series of conferences, symposia, and special committee meetings, (6) sponsored increasing numbers of Visiting Lectureships (foreign scholars, medical school lecturers, liberal arts speakers and high school visitations), (7) enlarged the placement service, and (8) promoted the Biological Science Curriculum Study (BSCS), a $1,200,000 project of the NSF involving the preparation and testing of textual material, laboratory, and field work directions, materials for gifted students, background materials for teachers, and a Teacher's Commentary at the 10th grade biology level.

3. *To N.A.S.–N.R.C.*, Division of Biology and Agriculture (N. D. Levine): More than 4,000 scientists serve on the more than 400 active committees of the NRC which has a membership of about 230.

The third annual meeting of the entire council was held in Washington, D. C. on March 24–26, 1960. It consisted of three panel-membership discussions: (1) *The Dynamics of Natural Resources* which indicated problems of the population explosion and the disposal of atomic wastes. (2) *Grants and Fellowships as Factors in the Development of Scientists* which noted that NSF and NIH fellows can and should be assigned teaching duties as part of their training, that a real shortage of teaching assistants exists, that about 60 percent of the first year NSF fellows are successful and that less emphasis should be placed on research grant assistantships with more on postdoctoral fellowships and the possibility that "block" research grants

could be made to individuals in research-administrative positions for 7 years (renewable after 5 years). (3) *Transportation and Patterns of Living* in which the great increase in per capita, intercity travel was discussed and the acute traffic problem considered.

The plenary session discussed the explosion in science and technology and its effects in the splintering of science.

4. *To the American Board of Microbiology* (N. D. Levine): At the first meeting of the American Board of Microbiology, consisting of five Fellows of the Academy and 7 representatives of selected scientific societies, it was decided to initiate a certifying program in the general field of public health and medical laboratory microbiology. A further revision of the rules and regulations of the Board was also decided upon.

The Rules and Regulations are in their fourth and probably final draft. The terms of the initial members of the board were set by lot at 3, 4, and 5 years. Your representative's term was set at 4 years.

The certifying program in the field of public health and medical laboratory microbiology is being prepared by the Standards and Examination Committee for Public Health and Laboratory Parasitology (N. D. Levine, Chr.). It is in its second draft and will be discussed by the Committee during the Los Angeles meeting. It is hoped that the first examination can be given in the spring of 1961.

A committee was organized to plan for programs of recognition of microbiologists at the B.S. level (primarily bacteriologists, but mycology and parasitology options are included). The Standards and Examination Committee has been asked to prescribe the requirements for the parasitology option. The "McKeown Committee" of the American Medical Association has recommended greater cooperation between physicians and scientists in the allied health professions. This, and the representation of the American Medical Association on the American Board of Microbiology, may overcome the objection by the medical profession that no one without the M.D. degree is eligible for specialty certification in medical bacteriology, parasitology, clinical chemistry, etc.

5. *To the Agricultural Research Institute* (E. E. Lund, Alt.): The Eighth (October 12–13, 1959) and Ninth (October 17–18, 1960) Annual Meetings of the NRC–ARI were attended. The first featured a program with the central theme "Research Outlook on Soil, Water and Plant Nutrients;" the second, "Interrelationships of Land-Grant Colleges, Government, Industry and Regulatory Agencies When Dealing With Agricultural Research Problems and Results."

Although parasitology was mentioned specifically on only a few occasions and primarily in the 1960 meeting, both programs treated subjects that pertained directly to parasitological problems. There appeared to be a lack of appreciation of the importance of parasitism in animal health and no recognition of its relationship to pasture problems, soil composition and practices, climatic factors, etc. It also was not considered in discussions of pesticides, chemical residues or intolerances. Steps should be taken to correct this situation.

The formation of a subcommittee of the Committee on Animal Health for the study of the relations of animal parasites could clarify parasitological problems. Your representative suggested this to Dr. Kruass and was advised that an appropriate appeal to the Presidents of the ARI and the Agricultural Board, the Chairman of NRC, or the Executive Secretary of the Division of Biology and Agriculture might produce results. Other Societies, such as the Society of Protozoologists, might wish to join in such efforts.

A motion that all representatives to the annual meetings of ARI (as of 1961) pay the customary costs for meals ($10.00) and a registration fee ($15.00) was approved at the Ninth Annual Meeting (1960). An expression of Council on the possibility that the American Society of Parasitologists pay part or all such expenses was requested by the delegate.

Council approved payment by the Society of the meal and registration fees for its representative.

6. *To the First International Conference on Trichinellosis* (B. Schwartz): The Conference convened in Warsaw, Poland on September 12, 1960. Nineteen countries were represented, including 11 delegates from the U. S. The entire proceedings of the Conference will be published by the Polish Academy of Science.

The program included reports on the worldwide incidence of *Trichinella spiralis*, the effects of ionizing radiations and ecological factors that influence its vitality and contribute to biochemistry, pathology, pathogenesis, immunology, clinical course and diagnosis. Perhaps the most important facet emphasized was sylvatic trichinosis, including the role of wild mammals in the maintenance of the parasite and in its dissemination to pigs, an important aspect of the epizootology and epidemiology of the infections.

September 14–15 were used for a field trip to Bialystok, Poland, where, on the average, more than 100 cases of clinical trichinosis have occurred for the past 10 or more years. From

Bialystok the group proceeded to the Bialowieza forest where intensive foci of trichinosis are present in small mammals. Visits to scientific institutions in both areas informed the group of work in progress which has bearing on the dissemination of trichinosis from wild animals.

The Conference approved the appointment of an International Commission on Trichinosis with headquarters at Wroclaw (C. Norwida Str. 29), Poland. Officers of the Commission are: Honorary President, Professor K. I. Skryabin (U.S.S.R.); President, Professor W. Stephanski (Poland) and Secretary, Professor Z. Kozar, (Poland). The Executive Committee of the Commission consists of a Chairman, Dr. B. Schwartz (U.S.A.); Vice-Chairman, Dr. S. E. Gould (U.S.A.); Secretary, Professor F. J. Kruidenier (U.S.A.); and Professor R. Lehmensick (W. Germany), Professor L. W. Orlow (U.S.S.R.) and Professor L. Guilhon (France).

The Executive Committee was charged with the responsibility of accumulating data on the epizootology and epidemiology of trichinosis from all over the world, with developing and encouraging the use of standardized techniques in cooperation with the World Health Organization, and to prepare or direct the publication of a comprehensive bibliography of trichinosis with summaries of recent, pertinent papers.

Dr. John Andrews (ARS–USDA) requested in behalf of Dr. Schwartz that the American Society of Parasitologists sponsor the International Commission on Trichinellosis. It was so moved and Council unanimously approved that the Society "sponsor the International Commission on Trichinosis with no financial obligation to the Society."

7. *To the inauguration of A. D. Holt as President of the University of Tennessee* (A. W. Jones): Your representative attended the ceremonies and tendered the congratulations of the Society to Dr. Holt.

The reports of the Representatives and Delegates were accepted and ordered placed on file.

IV. Reports of Affiliate Societies

1. *Southern California Parasitologists* (M. Voge): Five regular meetings were held during the past academic year. Members presented two stimulating movies concerning Africa as well as interesting papers for discussion. They were privileged to hear Sir Philip Manson-Bahr during his short visit to our area. Dr. L. R. Penner of the University of Connecticut, and Dr. Angela Taylor of the National Institute for Medical Research of London, England, have been working in Southern California during the past year and were guest speakers. A list of meetings is as follows:

(1) October 28, 1959. "Studies on Filarial Worms, using Phase-Contrast, Ultra-Violet and Fluorescent Microscopy" (Dr. Angela Taylor). (2) November 25, 1959. "Recent Work on Tropical Diseases Transmitted from Wild Animals to Man (Sir Philip Manson-Bahr). (3) January 28, 1960. "Recent Experiences in Africa"—with movie (Dr. Edward Wagner). (4) April 26, 1960. "What's WITH the Snails?" (Dr. L. R. Penner). (5) May 28, 1960. "Jumbo," a film about Africa (Dr. John Goodman).

The group vigorously supported a proposal to release Los Angeles County Pound animals to accredited research institutions. This was adopted as a county ordinance by a narrow margin.

The Local Committee on Arrangements for the joint meetings of the American Society of Parasitologists and the American Society of Tropical Medicine and Hygiene, composed of members of the Southern California Parasitologists, extends greetings to all who will visit us at the meetings, and trusts that everyone will have an enjoyable, profitable stay.

Dr. Edward Wagner of the School of Medicine of the College of Medical Evangelists was elected President of the Southern California Parasitologists for 1959–1960, and Dr. Jerrold Turner was elected Secretary-Treasurer for a 2-year term.

2. *Annual Midwestern Conference of Parasitologists* (D. T. Clark): The 12th Annual Midwestern Conference of Parasitologists, Dr. F. J. Kruidenier, Presiding Officer, was held at Purdue University on June 15–16, 1960. Fifty members attended, presenting 40 demonstrations.

The Conference program was coordinated with the International Symposium on Growth. The invited speaker, Dr. Paul Weinstein, presented a lecture on "Growth in Nematodes" to members of the Conference and visitors to the Symposium on Growth. Members of the Conference were conducted through the excellent laboratories and research facilities of the new Life Sciences Building at Purdue.

Dr. Norman D. Levine was elected Presiding Officer for 1960–61.

The 1961 meeting of the Conference will be held at Ohio State University in Columbus, Ohio. A symposium on immunity and host resistance will be held.

3. *The Helminthological Society of Washington* (L. Jachowski): Nine monthly meetings were held during the 1959–60 year as follows: No. 365 (October), Walter Reed Army Institute of Medical Research, Washington, D. C.; No. 366 (November), U. S. Department of Agriculture, Beltsville, Md.; No. 367 (December), Howard University, Washington, D. C. (a special session to hear Dr. Philip Manson-Bahr discuss "Wild Game and Man in Africa"); No.

368 (January), Catholic University of America, Washington, D. C.; No. 369 (February), University of Maryland, College Park, Md.; No. 370 (March), National Institute of Health, Bethesda, Md.; No. 371 (April), Johns Hopkins University, Baltimore, Md.; No. 372 (May), U. S. Department of Agriculture (Annual Picnic); No. 373 (October), University of Maryland, College Park, Md. Twenty-six technical reports were presented in addition to Sir Philip Manson-Bahr's discussion prior to the 373d meeting.

The fiftieth anniversary of the Helminthological Society of Washington was celebrated during the 373d meeting of the Society. A special number (27: 3) will be published in 1960 to contain the proceedings of this meeting.

Members, totalling 355, reside in 43 states, the Canal Zone, Puerto Rico and the District of Columbia and in 26 foreign countries. Thirty-three new members were elected during the year. Approximately one-half of the members belong to the American Society of Parasitologists.

Current officers are: President, G. W. Luttermoser; Vice-President, C. G. Durbin; Corresponding Secretary and Treasurer, E. M. Buhrer; Recording Secretary, L. A. Jachowski, Jr.

V. Reports of Special Committees

1. *Committee on Russian Translations* (K. C. Kates, Chr.; M. A. Doss, G. Anastos, E. C. Dougherty, L. Jacobs): The Committee has obtained translations of two Russian books in parasitology through Drs. W. J. Hargiss and I. G. Kagan. These will be published by the AIBS through grants from NSF. They are (1) Bychowsky, B. E. 1957. Monogenetic Trematodes. Their System and Phylogeny. 509 pp. and (2) Shikhobalova, N. P. 1950. Questions of Immunity in Helminthiasis. 184 pp.

Any member of a Member Society or Affiliated Society of AIBS may apply to the AIBS for translation services. The AIBS has no funds for translating purposes but will either apply for funds to an agency such as NSF, or request that the U. S. Department of Agriculture consider the inclusion of the translation in the program financed by funds available under Public Law 480.

Public Law 480 translations are financed by U. S. foreign currency funds in certain foreign countries under the supervision of the NSF and operating in cooperation with various departments of the government. ASP members may recommend needed translations through this program by application to AIBS or to the Director, Parasitological Laboratory, Animal Disease and Parasite Research Division, ARC, Beltsville, Md. Special forms will be furnished for application through AIBS upon request to Dr. Francis Harwood, Director of Publications, AIBS, 2000 P St., N. W., Washington 6, D. C.

Four translations of Russian texts have been contracted for through Public Law 489 during the past year. Published translations will be made generally available through the Office of Technical Services, U. S. Dept. of Commerce, Washington 25, D. C. The texts are:

(1) Skrajabin, K. I. *et al.* 1954. Trichostrongyloidea of Animals and Man. Osnovy nematodologii, v. 3. 684 pp. Moscow. (Now available.)

(2) Spasskii, A. A. 1951. Anoplocephalata Cestodes of Domestic and Wild Animals. Osnovy tsestodologii, v. 1. 735 pp. Moscow.

(3) Skrajabin, K. I. *et al.* 1952. Strongylata. Opredelitel' paraziticheskikh nematodologii. v. 3. 890 pp. Moscow.

(4) Antipin, D. N. *et al.* 1956. Parasitology and Invasive Diseases of Livestock. 479 pp. Moscow.

It was suggested that (1) Council may wish to make the Committee on Russian Translations a *Standing Committee* instead of a *Special Committee* and (2) that members of the Society make available any extensive private translations to the Office of Technical Services so that they may be generally available.

Council approved both suggestions.

2. *Committee on Public Health Laboratory Problems, APHA* (M. M. Brooke, Chr.): The APHA Committee on Public Health Laboratory Problems devoted its attention to the laboratory programs of state and regional public health associations, finding that few or no programs exist and that programs presented attract poor attendance.

Dr. M. Scherago, Chairman of the APHA Committee on Public Health Problems, has indicated that during the coming year its members may be more concerned with the program services of State Health Department laboratories. The ASP representative has suggested, in the event of this study, that attention be given to offering serologic services for toxoplasmosis in state laboratories. Four or five states currently offer such service but many are under pressure from ophthalmologists and other physicians to develop the service. It was suggested to Dr. Scherago that his committee might review the needs for the tests and assist in obtaining financial support for them.

3. *Career Booklet Committee* (D. V. Moore, Chr.; G. L. Hoffman, R. M. Cable, D. L. De-

Guisti, C. G. Goodchild, D. A. Porter, R. E. Thorson, M. Ferguson): It was hoped that the final revision of the Career Booklet can be completed and edited by the entire committee very soon. The committee requested that Council consider the financial aspects of publication of the booklet.

The number of inquiries for material such as this has increased and the committee has on hand a file of such requests.

4. *Journal Index Committee* (W. S. Bailey, Chr.; M. A. Doss): See report of the Custodian. INDEX II is finished and will be distributed early in December 1960. It is available to members at a post-publication price of $7.50.

5. *Committee on Annual Meetings* (N. D. Levine, Chr.; D. P. Furman, C. M. Herman, G. W. Hunter III, D. L. Price): The report of this Committee is submitted in the form of 27 proposals for Council's consideration. The proposals are based on a questionnaire submitted to the membership of the Society by the committee and which was answered by 39.2 percent of the members. Proposals and Council vote are as follows:

The committee recommends:

(1) That the present arrangement of a luncheon followed by the business meeting be continued. *AYE.*

(2) That the Supplement containing abstracts of papers presented at the annual meeting and abstracts of papers presented by title be continued. It recommends that the latter practice be discouraged. *AYE.*

(3) That the Society refuse to publish abstracts containing the first description of a new taxon. *AYE.*

(4–5) That several simultaneous regular paper sessions be scheduled, organized according to subject, dependent upon the number of papers submitted, and avoiding conflict if possible. *AYE.*

(6) That papers be limited to 10 minutes. *AYE.*

(7) That no more than 4 full reports be scheduled per hour with a five minute allowance for discussion. *AYE.*

(8) That the appended "suggestions for" giving reports and preparing lantern slides be sent to the members with the call for papers. *AYE.*

(9) Enlargement of the demonstration sessions to also include a special subject of general interest with old as well as new material dealing with it. *AYE.*

(10–11) The scheduling of an informal discussion session with a moderator and that this be recorded for later editing and publication. *NO.*

(12–15) That a review type, informal symposium be held at the next meeting, that the subject be on (a) the laboratory diagnosis of parasitic infections, (b) the bases of host specificity, or (c) epidemiology of animal parasites, that the papers be published, and that the meeting be prolonged to accommodate this if necessary. *AYE.*

(16) That the Society suggest to the American Society of Zoologists that the two societies collaborate at the 1961 meeting in presenting a refresher course on Recent Advances in Parasitology. *AYE.*

(17) That a series of invited lectures be established to deal with aspects of parasitology not ordinarily covered at the meetings as plant nematology, insect galls, hyperparasitism, biological control of insect pests, insect pathology, predaceous fungi, parasitic copepods, parasitic molluscs, etc. *AYE.*

(18) That the Society occasionally apply for a grant from an appropriate agency (NSF, NIH, etc.) to bring a distinguished foreign parasitologist to this country to deliver an evening lecture at the annual meeting and visit laboratories of parasitology in this country. *AYE.*

(19) That a Program Officer be established in the Society, that his term be staggered with that of the Secretary (2 years) and that he work with the Local Representative, the Secretary and others in charge of meeting arrangements to correlate the programs. This would necessitate a change in the By-Laws but could be appointive (Presidential?) pending such change. *AYE.*

(20–21) That a special room near the meeting be assigned to the Society for talking, coffee, a bulletin board, etc. *AYE.*

(22) That an informal job-placement service be established at those meetings where there is no such service. This could consist of a special bulletin board as recommended in # 20–21. *AYE.*

(23) That a separate meeting be arranged by the Society for 1962 with the aim of making such meetings periodic thereafter if they prove feasible. The timing should not conflict with that of AIBS, AAAS, or ASTM&H meetings. *AYE.*

(24) That a special committee be appointed to consider the desirability of establish-

ing prizes, awards and citations of various kinds and especially of establishing a prize for the best paper given by a student at the annual meeting. *AYE.*

(25) That suggestions made by members responding to its questionnaire be given serious consideration by Officers and Council of the Society. *AYE.*

(26) That recommendations 1, 2, 4, 5, 6, 7, 8, 9, 10, 12, 13, 15, 16, 19, 20, 21, 22, 23, and 25 be put into effect before the next meeting. *NO.*

(27) That this report and its appendices be published *in toto* in the Journal or that an announcement be made that the complete report can be obtained from the Secretary. *NO.*

An added report by N. D. Levine briefed Council on the meeting of officers of the animal sciences societies which met at Oklahoma State University, Stillwater, during the AIBS meeting there on August 28, 1960.

The International Congresses of Zoology were discussed by Dr. Witschi who announced that the next Congress will be held in Washington, D. C., August 21–27, 1963. One section on parasitology was held during the London Congress.

The report of the Policy Committee of the American Society of Zoologists was discussed by C. L. Prosser, President-Elect of ASZ. The work of the committee has resulted in (1) the establishment of an educational program, one part of which is a Refresher Course at each ASZ meeting and (2) the establishment of a number of divisions in ASZ.

Dr. Prosser asked if the group felt that there was a place for ASZ as an integrating organization between specialty societies (such as ASP) and the AIBS. The general feeling was that there is. Various ideas were suggested, some of which already have been put into effect. They include: (1) ASZ promotion of inter-scientific programs. (2) A cooperative secretarial service. ASZ investigated the use of the AIBS business service but felt that it was not financially feasible because of cost and that it was preferable to spread the work among colleagues. (3) Summer institutes, already started. (4) ASZ applications for grants in behalf of member societies. (5) Publication of a general zoology journal with emphasis on the publication of symposia and refresher courses. Plans for this are well along. (6) ASZ sponsorship of regional meetings of which five now exist. (7) Sponsorship of meetings of animal science biological societies by themselves. It would be possible to start this during the 1962 period.

Specifically, and in relation to the report of the Annual Meeting Committee, it was suggested that ASP cooperate in the presentation of a refresher course in Recent Advances in Parasitology during the course of an annual meeting, that ASP consider sponsoring a summer institute, and that ASP consider the possibility of a separate meeting of animal biology societies.

Council approved the development of the ideas of the refresher course and the summer institute in general. It further approved the general concepts of cooperation with ASZ and the inter-society council of representatives.

6. *Committee on Meetings in 1962, 1964, and 1965* (G. W. Hunter, III, Chr.; R. M. Cable, F. C. Goble, A. I. Goldsby, A. McIntosh): The committee recommends the following schedule of meetings for the period 1961–1965:

1961: With AIBS at Purdue (the Society is committed to this).

1962: Washington, D. C., mid-June, with the Helminthological Society of Washington, and, possibly, the Annual Midwestern Conference of Parasitologists. The Committee recommends that the meeting be held at the Shoreham Hotel (if available) and that a symposium be substituted on this occasion for the usual demonstration session.

1963: The Society is committed to a joint meeting with the American Society of Tropical Medicine and Hygiene in Chicago, Ill. (November).

1964: With AIBS in August at the University of Colorado at Boulder, Colorado.

1965: With the Annual Midwestern Conference of Parasitologists at Ashville, N. C. in mid-June. It is suggested that a symposium be substituted for the demonstration session.

Council approved the tentative schedule suggested by the Committee.

7. *Committee on the Nominations of Officers* (R. E. Thorson, Chr.; D. Fairbairn, F. A. Ehrenford, F. C. Friedl, J. A. Scott): The Committee believes that a change in the method of selecting officers of the Society is highly desirable and that generally, two slates of officers and a nominating committee should be nominated by a committee of members who are not elected officers of the Society. A proposal to amend Sections 2 and 3 of Article IV, Administration, of the Society's By-Laws is presented.

Council approved the suggestion and ordered the Society vote on the amendment during 1961. The proposal will be submitted to the members.

Council approved a motion to instruct the 1961 Nominating Committee to select a slate of two nominees for the offices of President-Elect, Vice-President, and Councilors, and at least one nomination for each of the remaining offices to be filled.

Council approved a motion that nine nominees to the 1962 Nominating Committee be

presented at the Annual Business Meeting in 1961 and that the five nominees receiving the highest number of votes shall form the Nominating Committee for 1962 in accord with the procedure recommended by the Committee on the Nominations of Officers.

8. *Committee on International Organization* (F. J. Kruidenier, Chr.; B. Schwartz, T. W. M. Cameron, E. H. Sadun, R. D. Stoner, R. Rausch): This committee was designated by the Secretary to help him investigate the proposal by Z. Kozar, President of the Polish Society of Parasitologists, that an international organization of parasitologists be created. All members of the committee attended the 1st International Conference on Trichinellosis in Warsaw, Poland (see above). Further opinions were requested from Dr. A. M. Fallis, active member of the society from Canada and J. Baer, honorary member, Neuchatel, Switzerland, in order to gauge opinions in areas other than the United States. An opinion of Council was requested prior to the Warsaw meeting.

Council indicated interest in the proposal. Dr. Fallis felt that Canadian colleagues agreed that an international organization would be "most valuable and interesting," and Dr. Baer believed that the general European and British reaction would be favorable. Dr. Kozar provided a summary of his questionnaire which was sent to parasitologists throughout the world and in which answers from parasitologists of about 20 countries (24 from the U. S.) were uniformly favorable to his proposals (detailed analysis submitted to Council). The Society of Protozoologists expressed interest and appointed F. J. Kruidenier its representative to the organizing conference in Warsaw.

After considering numerous pertinent factors the committee agreed on certain fundamental concepts that would be essential to the success of an international organization. These have been outlined tentatively in the proposal "International Federation of Parasitologists" (submitted to Council). The federation would consist primarily of affiliated societies and be administered by a commission with an executive committee. The commission would be composed of representatives selected by and for each member society. The executive committee would be chosen from its members by the commission, with its chairman and secretary serving in like capacities on the commission. All members of the federated societies would automatically become members of the federation. All communication of the federation with such members would be through the routine channels of the respective societies.

Each participating society would thus retain all rights of contact with its own members and it is proposed that such a system would automatically prorate most of the costs of the federation to individual societies and minimize international financial obligations. It would concurrently minimize difficulties of international monetary restrictions or potential exchange losses to the societies.

Tentative proviso is made for the membership of individual parasitologists who may not find it feasible to belong to one of the federated societies by reasons of geographical or other physical factors. Such membership would have to include realistic fee levels which would support them adequately. Strong arguments against and in support of this feature have been presented to the ASP Committee and it might be necessary to leave final determination to the first elected commission.

Affiliation of the proposed federation with one of several international organizations has been considered but not investigated as yet. Three such groups have been mentioned: The International Union of Biological Sciences, The International Congress of Zoologists, and The International Council of Scientific Unions.

The advantages inherent to an international organization have been emphasized variably. These include the correlation of the increasing number of splinter "international conferences" into a single, correlated unit series, a cooperative broadening of parasitological abstracting services, the stimulation and correlation of international efforts to control parasitic diseases with authoritative sponsorship, the systematization of the storage of type specimens and equitable, official distribution of available paratype specimens, and other lesser items.

The proposals have the unqualified approval of Dr. Jean Baer (Switzerland) and have been approved by Dr. Kozar, President of the Polish Society (for submission to the Polish Parasitological Society) and Dr. Ettore Biocca, President of the Italian Society of Parasitologists and his executive council (for submisison to the Italian Society). They are being considered by the newly forming society in England and are being sent to the Japanese Society of Parasitologists.

Council approved, in general, the steps taken thus far and authorized the appointment of a committee to continue the investigation of the potentials and advantages of the international proposals.

9. *Committee on Business Operations* (L. Jacobs, Chr.; G. F. Otto, A. C. Walton): The report of this committee is in the form of a "Proposal to The American Society of Parasitologists" summarized as follows (submitted *in toto* to Council in October, 1960): The American

Society of Parasitologists stands now before a challenge as to its capacity to serve the interests of all parasitologists. The Journal of Parasitology should be expanded to provide a medium for the publication of more of the papers now published elsewhere. The business management of the Society should be centralized and simplified. The Society should grow and meet its responsibilities by a frank recognition of its present status and a vigorous plan for future development.

Demand for expansion of the Journal has been expressed by a large number of the members of the Society. It should contain more experimental papers. The costs, to authors, of long papers or those containing many tabular data are too high and result in the loss of material that the Journal should publish.

The Editor is submerged in many mechanical details related to his office. The Secretary and Treasurer carry large burdens of detail work. The Society is not only depending on the generosity of these functionaries but of their institutions, which donate space and secretarial help. We have a compelling problem in regard to the management of our custodial office.

The Journal cannot be doubled without providing adequate help for the Editor. Increased membership and subscriptions will also increase the burdens of Secretary and Treasurer. Because of these interrelations, the Committee arranged a meeting in Washington, D. C., at AIBS headquarters, where officers and immediate past officers of the Society could discuss these matters with the committee.

The committee envisions doubling the Journal of Parasitology by 1963, with the hastening step of the removal of all costs to authors (except in cases where research funds from national granting agencies are made available for this purpose). We would announce that the Journal is interested in receiving more manuscripts in the fields of experimental and physiological parasitology. This should also double the growth rate of membership and subscriber lists so that a total of 2,500 would be achieved by 1963.

Such growth will require centralized offices for performing redactory and business management services. The American Institute of Biological Sciences offers these services. Their program provides maximal cost estimates safeguards and will probably be revised downward. These rates are incorporated into our proposed Annual Budget for expansion as follows:

Costs:	
Printing of Journal (1200 pp. with allowance for increased costs)	$34,400.00
Redactory Services (AIBS, $2.00/page)	2,400.00
Business Management (AIBS, $1.75/name—2,500 names)	4,375.00
Officer Travel	400.00
Offices Expenses	
Secretary	150.00
Treasurer	100.00
Editor	400.00
Editor's Secretary	300.00
AIBS Membership Dues	1,250.00
Total Costs	$43,775.00
Revenues:	
Dues (at $15.00 each for 1,250 members)	
Subscriptions ($16.50 each for 1,250)	$39,275.00
Other receipts (sales of back issues, advertising, membership lists, etc.)	4,500.00
Total Revenues	$43,775.00

We would justify this very substantial increase in the budget and its cost to individual members and subscribers by making the following points:

(1) There will be a 100% increase in return of information in the form of a doubled Journal for an 87.5% increase in cost.

(2) This expansion *cannot* take place without the use of a redactory service to ease the editor's burden. AIBS services will leave the Society *in absolute control of the Journal:* we will buy copy-preparation, galley-reading, page-make-up, etc., all the menial, time-consuming chores that now plague the Editor.

(3) Business management costs are being paid now in the offices of the Treasurer and Secretary. We would pay AIBS for dues and subscription collection, maintenance of membership and subscriber lists, billing for advertising, sales of membership lists (on the basis of secretarial approval), etc. *but fiscal management will remain in the hands of the Society.* The Society will have a permanent, unchanging address.

The proposed increased cost of membership in the Society is not excessive. Dues in most professional societies are generally from $12–$15. Salaries and the cost of living have gone up.

Graduate students, for example, now receive four times as much as in 1933. We cannot expect to continue the *status quo.* We have received such a large Journal in the last few years only because we have been practising deficit financing, paying in the next year for two issues of the previous year.

The values returned to members will be multiple: (1) Extensive subject coverage in an oustanding journal; (2) improved quality of the Journal because the Editor will be able to devote his time to *editing* rather than redactory "scut" work; (3) speedy publication; (4) liberalization of charges for publication; (5) relief of officers from detail work; (6) a central, stabile business office for routine affairs.

It must be emphasized that we would merely buy services from AIBS at the lowest possible price, without subservience to AIBS. Savings will be passed back to the Society because AIBS is not a profit-making organization.

Alternately, the Society can continue to struggle along, losing the battle of inflation, members to other organizations and privately published journals or to splinter groups. The opportunity is here and we either take it or undergo contraction in the future. There is no standing still: we either grow with vigor and purpose or we decline.

10. *Service Officers' Analyses of Society business affairs:* (F. J. Kruidenier, M. J. Ulmer, E. R. Becker): Much of the subscription business of the Society is accomplished through agents, dealing in block library or institutional orders of various publications and the agents receive, approximately, a 10 percent commission from the sale price of the publications. This reduces the proposed income (Business Operations Committee) on 1500 subscriptions (plus 1500 memberships) to $38,038 or less, depending upon the ratio of agent: non-agent subscriptions. This increases the amount that the Society must derive from other sources to $5,737 if a budget balance of income and expenditure is to be realized in an activation of the suggested program. A very substantial increase in the receipts which the Society realizes from sources other than dues and subscriptions would be necessary to meet the tentative budget. The average net receipts of this sort for the past 10 years has been appreciably less than $1,000 (exclusive of charges to authors). A partial summary of Society business for the past 10 years is as follows:

	JOURNAL COST				AIBS	INCOME	
Year	Pages	Press Charges	Editorial Cost	Billing (Subscr.)	Cost	Members	Subscriptions
1950	674	$9,020	$266	$320	$511	628 ($6430)	817 ($5701)
1951	668	9,419	272	238	496	654 (2215)	885 (5493)
1952	661	9,808	712	349	500	672 (4526)	921 (6862)
1953	743	11,125	589	521	524	702 (4803)	973 (6862)
1954	795	12,312	189	371	543	737 (4889)	987 (6701)
1955	715	11,898	290	330	650	802 (5302)	1082 (8344)
1956	720	12,616	557	379	1197	819 (5668)	1119 (8344)
1957	791	14,167	289	318	833	844 (5554)	1203 (7940)
1958	728	14,348	264	423	761	934 (5953)	1022 (7571)
1959	747	15,328	344	434	913	964 (8033)	1110 (10168)

Council approved motions (1) that the Editor's budget be increased to $3,500 for a period of one year for the operation of the Journal as he judges best, and (2) that the Treasurer's budget be increased to $1,500.

The Council approved a motion recommending to the Society that dues be increased to $15.00 per year for full members and $10.00 per year for members whose student status is certified by the Chairmen (Heads) of the departments in which they are studying, and that the moneys be used for expanding the size and scope of the Journal of Parasitology and to reduce author charges and the time of publication of acceptable reports.

VII. Old Business

1. *The Society ballot on subscription to Helminthological Abstracts:* The Secretary reported that a total of 561 members voted this ballot. Of these, 330 members indicated a definite interest in ordering Helminthological Abstracts through the American Society of Parasitologists at the specified 50 percent discount, while 231 indicated unwillingness to order the publication regardless of the discount. Approximately one-half abstained.

Council indicated without formal vote that it was unwilling to recommend Society subscription of 1,000 copies of Helminthological Abstracts for distribution by the Society under these circumstances.

VIII. New Business

1. *Demonstration sessions of the Annual Meetings:* The secretary pointed out that only about 10 demonstrations had been submitted for the 1960 meeting until letters to several members stimulated the submission of a sufficient number to make the session feasible.

2. *The status of foreign ASP members in AIBC:* The secretary outlined correspondence with Dr. Hiden T. Cox, Executive Director, AIBS, in which it was proposed to enroll foreign members of American Societies in AIBS for distribution of the AIBS Bulletin and Newsletter through foreign distribution agencies.

Council approved enrolling overseas members of ASP for receipt of full membership benefits in AIBS.

3. *Proposal to hold a joint symposium in 1961 with the Wildlife Disease Association:* The secretary read a letter from Dr. C. M. Herman, President of the Wildlife Disease Association, requesting that an informal discussion symposium be held jointly with the Wildlife Disease Association during the 1961 meetings with AIBS at Purdue. Dr. Herman proposed that the symposium subject be a discussion of either *Parasites of Waterfowl*, or *Parasites of Deer and Other Cervidae*. He further suggested that Dr. Norman D. Levine be requested to act as chairman or discussion leader.

Council determined that Dr. Levine will not be able to attend the 1961 meetings because of conflicting meetings that he is pledged to attend.

Council approved of Dr. Herman's suggestion otherwise and directed the secretary to correspond further with Dr. Herman concerning the plans with the reservation that the suggested symposium would have to be held concurrently with other sessions or in the evening.

4. *Communication from the National Medical Library of Czechoslovakia:* The library (Prague, Czechoslovakia) publishes a bibliography "The Annual of Czechoslovak Medical Literature." Dr. Josef Navaratil, Librarian, asks if ASP wishes to receive the bibliography with a view "to develop friendly contact with the medical libraries abroad, to establish an exchange of publications with them and perhaps, later, to reach a closer cooperation still."

Council directed the secretary to thank Dr. Navaratil, informing him of the similarity of the desires of the American Society with those expressed by him, but to inform him also that the society has no facilities which would make such an exchange possible.

5. *Communication from the Squibb Institute:* The Society is informed that it has been selected by the Special Libraries Association, Science-Technology Division, to be included in its publication Scientific Meetings, published three times each year.

Council directed the secretary to thank the Institute and to further the necessary information to Mrs. Mary S. Klein as requested.

6. *Senate Bill S3570* (L. J. Olivier): Resolved: The Council of the American Society of Parasitologists notes that there has been introduced for consideration by the Senate of the United States of America a bill (S3570) which deals with the care of animals used for experiments and tests by instrumentalities and agencies of the United States Government and by recipients of grants from the same.

The Council recognizes that experimental animals should be given humane treatment at all times and that vigilance in enforcing humane procedures is necessary.

The Council also recognizes that the intent of the Bill is good to the extent that it seeks to protect the welfare of experimental animals.

However, the Council wishes to go on record as opposed to the substance of the Bill. The Council is firmly convinced that the provisions of the bill stated in Sections 2 and 9 are unnecessary and undesirable. Moreover, the Bill would tend to severely hinder medical research and essential research so important to progress.

The Bill is unnecessary since existing law and custom already require humane treatment of experimental animals and provide adequate mechanisms for enforcing these requirements.

The Bill is undesirable since it singles out laboratories of the United States Government, and the outstanding laboratories it subsidizes through grants, for supervision and control while it does not affect many other laboratories.

The Bill would hinder research involving animals since the requirements for certification, for filing of "project plans," for making annual reports, and for inspection by the department of Health, Education and Welfare would divert precious time and money from the conduct of research.

For these reasons, the Council, in the name of 1,100 members of the American Society of Parasitologists urges the defeat of Senate Bill S3570.

Council directed the secretary to determine the best means of informing the members of Congress of the undesirability of Bill S3570.

7. *President-Elect J. M. Andrews* suggested that the Society might desire the dissemination of information concerning events of scientific nature reported at its meetings through

proper channels and offered such services by the Information Office of the National Institute of Allergy and Infectious Diseases, NIH.

Council approved inviting the Information Officer of the National Institute of Allergy and Infectious Diseases to its national meetings for this purpose.

8. *Liaison with Affliated Societies:* The Secretary was directed to include Council Representatives of affiliate societies as recipients of all communications to Council although their status, as determined by the By-Laws of the American Society of Parasitologists, is that of non-voting members of the Council.

9. *Meetings for 1963* (G. F. Otto): No report.

10. *Nominees to Active Membership in the Society* (F. J. Kruidenier, Sec.): The applications for active membership of the following individuals were presented: Semar, Richard W.; Rogers, William E.; Deschamps, Guillermo D.; Soulsby, E. J. L.; Prouty, James L.; Crandall, Richard B.; Ma, Solomon; Ostlind, Dan A.; Bennett, Gordon Frager; Cherrie, Anna L.

Council unanimously voted acceptance of the candidates.

11. *Election of Assistant Editors:* The terms of Drs. G. M. Kohls and C. G. Goodchild expire in 1960. On recommendation of the Editor, Council elected G. M. Kohls and K. C. Kates to 4-year terms of office (1961–1964).

12. *Appointments to Standing Committees and Special Committees and of the Representatives and Delegates of the Society:* Council unanimously authorized the President of the Society to make the necessary appointments.

The Council voted to adjourn at 1:30 A.M., November 2, 1960.

Respectfully submitted,
/s/ Francis J. Kruidenier, *Secretary*

AMERICAN SOCIETY OF PARASITOLOGISTS

Thirty-Fifth Annual General Business Meeting, November 3, 1960

The General Business Meeting of the Society was called to order by President Brown at 1:45 P.M. following the annual luncheon held in the Hotel Biltmore, Los Angeles, California.

1. The status of the membership of the Society was summarized briefly by the Secretary. Members were notified that non-member subscription rates to the Journal had been raised to $16.50 (foreign) as of January 1, 1961, and that the charges to authors for publication in the Journal had been liberalized so that the Society now assumes all such costs up to $250.00 and one-half of costs between that and $300.00. The status of progress concerning the proposal by the Polish Society of Parasitologists that an international organization of parasitologists be formed was discussed. Council's approval of the progress to date and its directive to investigate the potentials of the affiliation of the American Society in the world organization was noted.

2. The financial status of the Society was reviewed.

3. A brief report on the Journal was presented. The Editor described the plans for use of the two column format and called attention to the appreciably increased pagination of the December 1960 (v. 46, No. 6) issue. Plans and hopes for future expansion were described. It was stressed that success depends upon the submission by members of a greater number and variety of reports for publication in the Journal.

4. The affairs of the Custodian's office were reviewed. The status of indexing volumes 27–40 (INDEX II) was presented. Prepublication subscriptions will be in the hands of members by December. The postpublication price of $7.50 was announced. A price of $5.00 was set for new members of the Society.

5. Future meetings of the Society were listed as follows: 1961, with AIBS in late August-September at Purdue University, Lafayette, Indiana; 1962, with the Helminthological Society of Washington in Washington, D. C. in mid-June; 1963, with the American Society of Tropical Medicine and Hygiene in Chicago, Illinois, on Nov. 6–9; 1964, with AIBS at the University of Colorado in Boulder, Colorado, during late August–September; 1965, with the Annual Midwestern Conference of Parasitologists in mid-June at Asheville, N. C.

6. Dr. D. V. Moore, in the absence of the Chairman of the Nominating Committee R. V. Rozeboom, presented the following slate of nominees for the elective offices of the Society:

President-Elect, L. A. Stauber; Vice-President, G. H. Ball; Treasurer, M. J. Ulmer; Council Members at Large (4 year terms), R. McGhee, J. A. Scott (A. M. Fallis was proposed to succeed G. H. Ball on Council in the event that the latter was elected to Vice-President).

The Secretary was instructed by the Society to cast one ballot in its behalf for the slate of nominees listed above.

7. The Society elected D. L. DeGiusti as Chairman of the Nominating Committee for 1961.

8. L. J. Thomas, reporting as Chairman of the Committee on Honorary and Emeritus Members, nominated Dr. Emile Ronbaud to Honorary Membership and Drs. W. H. Wright and H. E. Meleney to Emeritus Membership.

The above nominees were elected unanimously by the Society.

9. President Brown briefly discussed the desirability of and the necessity for expansion of the Journal of Parasitology and the Society. Following the discussion the secretary was requested to present the following motion to the Society in behalf of Council: Resolved that the membership dues to the American Society of Parasitologists be increased to $15 per year as of January 1, 1961, for all members except those actively engaged in scholastic work leading to academic degrees, and that dues to the latter as shall obtain certification of their student status from the respective heads or chairmen of the departments in which they are working shall be $10 per year. The said increase in dues is to be applied to expanding the Journal of Parasitology and expediting the expansion of the Society.

An invitation for discussion of the resolution from the floor was requested. It was moved and seconded that the resolution be adopted and the Society voted unanimously in favor of the resolution.

10. R. V. Bangham, Chairman of this Resolutions Committee, proposed the following resolutions:

(1) Be it resolved that the American Society of Parasitologists express its appreciation to the local committee on arrangements and especially to the co-chairmen Drs. Gordon H. Ball and John F. Kessel.

(2) Be it resolved that the American Society of Parasitologists express to the host organizations, and in particular to the Southern California Parasitologists, its appreciation of their help in organizing this meeting and contributing to its success.

(3) Be it resolved that the American Society of Parasitologists express its appreciation to the Hyland Laboratories for their generous sponsorship of various activities during this meeting.

(4) Be it resolved that the American Society of Parasitologists express its appreciation to the Biltmore Hotel for the highly satisfactory arrangements for these meetings and the many ways in which it contributed to the comfort and convenience of the participants in the meeting.

The report was unanimously adopted by the Society.

11. Dr. N. D. Levine announced that the First International Conference on Protozoology and the Thirteenth Annual Meeting of the Society of Protozoologists will be held August 22–3, 1961, in Prague, Czechoslovakia.

National Science Foundation and National Institutes of Health travel grants have been applied for which would cover round trip coach fares for scientists wishing to attend the meeting. Pending approval of the grants, scientists desiring to attend under the grants should write to Dr. William Trager, President, Society of Protozoologists, The Rockefeller Institute, New York 21, New York, before December 15, 1960, giving a brief summary of education, training and experience and the subject of the report which they wish to present.

Applicants need not be members of the Society of Protozoologists: all applications will be judged individually.

12. President Brown called the attention of the Society to the Henry Baldwin Ward Medal, on display with the Parke, Davis and Company exhibits. He described the beauty of the medal, the meticulous care that had gone into its design and the very real debt of gratitude that the Society owes to Dr. Justus F. Mueller for the long, arduous work that went into the creation of the medal.

13. President Brown expressed his appreciation to the Society for the opportunity which it had given him to serve it as President for 1960. He presented the President's gavel to Dr. Justin M. Andrews, his successor and President of the Society for 1961.

The Society adjourned at 2:15 P.M.

Respectfully submitted,
/s/ Francis J. Kruidenier, Secretary.

IN MEMORIAM

Paul S. Bartsch	August 14, 1871—March 24, 1960	Charter Member
Herbert S. Clark	November 11, 1877—November 8, 1960	Charter Member
Thurlow C. Nelson	September 22, 1890—September 12, 1960	Charter Member

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No. 1, February 1960 ..... mailed February 18, 1960
No. 2, April 1960 ..... mailed April 13, 1960
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